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(54) Title: VASCULAR ENDOTHELIAL GROWTH FACTOR-X

(57) Abstract

There is provided a novel vascular endothelial growth factor, herein designated VEGF-X, in addition to the nucleic acid molecule encoding it, a host cell transformed with said vector and compounds which inhibit or enhance angiogenesis. Also provided is the sequence of a CUB domain present in the sequence of VEGF-X which domain itself prevents angiogenesis and which is used to treat diseases associated with inappropriate vascularisation or angiogenesis.

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VASCULAR ENDOTHELIAL GROWTH FACTOR-X

The present invention is concerned with a novel
vascular endothelial growth factor (VEGF) herein
5 designated "VEGF-X", and characterisation of the
nucleic acid and amino acid sequences of VEGF-X.

Introduction

10 Angiogenesis involves formation and proliferation of
new blood vessels, and is an essential physiological
process for normal growth and development of tissues
in, for example, embryonic development, tissue
regeneration and organ and tissue repair.
15 Angiogenesis also features in the growth of human
cancers which require continuous stimulation of blood
vessel growth. Abnormal angiogenesis is associated
with other diseases such as rheumatoid arthritis
psoriasis and diabetic retinopathy.
20 Capillary vessels consist of endothelial cells which
carry the genetic information necessary to proliferate
to form capillary networks. Angiogenic molecules
which can initiate this process have previously been
25 characterised. A highly selective mitogen for
vascular endothelial cells is vascular endothelial
growth factor (VEGF) (Ferrara et al., "Vascular
Endothelial Growth Factor: Basic Biology and Clinical
Implications". Regulation of angiogenesis, by I.D.
30 Goldberg and E.M. Rosen 1997 Birkhauser Verlag
Basle/Switzerland). VEGF is a potent vasoactive
protein which is comprised of a glycosylated cationic
46-49 kd dimer having two 24 kd subunits. It is
inactivated by sulphhydryl reducing agents and is
35 resistant to acidic pH and to heating and binds to
immobilised heparin.

VEGF-A has four different forms of 121, 165, 189 and 206 amino acids respectively due to alternative splicing. VEGF121 and VEGF165 are soluble and are capable of promoting angiogenesis, whereas VEGF189 and VEGF206 are bound to heparin containing proteoglycans in the cell surface. The temporal and spatial expression of VEGF has been correlated with physiological proliferation of the blood vessels (Gajdusek, C.M., and Carbon, S.J., Cell Physiol., 139:570-579, (1989)); McNeil, P.L., Muthukrishnan, L., Warder, E., D'Amore, P.A., J. Cell. Biol., 109:811-822, (1989)). Its high affinity binding sites are localized only on endothelial cells in tissue sections (Jakeman, L.B., et al., Clin. Invest. 89:244-253 (1989)). The growth factor can be isolated from pituitary cells and several tumor cell lines, and has been implicated in some human gliomas (Plate, K.H. Nature 359:845-848, (1992)). The inhibition of VEGF function by anti-VEGF monoclonal antibodies was shown to inhibit tumor growth in immune-deficient mice (Kim, K.J., Nature 362:841-844, (1993)).

VEGF proteins have been described in the following patents and applications all of which are hereby incorporated by reference EP-0,506,477, WO-95/24473, WO-98/28621, WO-90/13649, EP-0,476,983, EP-0,550,296, WO-90/13649, WO-96/26736, WO-96/27007, WO-98/49300, WO-98/36075, WO-98/840124, WO-90/11084, WO-98/24811, WO-98/10071, WO-98/07832, WO-98/02543, WO-97/05250, WO-91/02058, WO-96/39421, WO-96/39515, WO-98/16551.

The present inventors have now identified a further vascular endothelial growth factor, designated herein as "VEGF-X", and the nucleic acid sequence encoding it, which has potentially significant benefits for the treatment of tumours and other conditions mediated by inappropriate angiogenic activity.

Summary of the Invention

In the present application, there is provided a novel vascular endothelial growth factor, herein designated "VEGF-X", nucleic acid molecules encoding said growth factor, an expression vector comprising said nucleic acid molecule, a host cell transformed with said vector and compounds which inhibit or enhance angiogenesis. Also provided is the sequence of a CUB domain present in the sequence of VEGF-X which domain itself prevents angiogenesis and which is used to treat diseases associated with inappropriate vascularisation or angiogenesis.

Detailed Description of the Invention

Therefore, according to a first aspect of the present invention there is provided a nucleic acid molecule encoding a VEGF-X protein or a functional equivalent, fragment, derivative or bioprecursor thereof, said protein comprising the amino acid sequence from position 23 to 345 of the amino acid sequence illustrated in Figure 10. Alternatively, the nucleic acid molecule of the invention encodes the complete sequence identified in Figure 10 and which advantageously includes a signal peptide to express said protein extracellularly. Preferably, the nucleic acid molecule is a DNA and even more preferably a cDNA molecule. Preferably, the nucleic acid molecule comprises the nucleotide sequence from position 257 to 1291 of the nucleotide sequence illustrated in Figure 9. In a preferred embodiment the nucleic acid is of mammalian origin and even more preferably of human origin.

In accordance with the present invention a functional

equivalent should be taken to mean a protein, or a sequence of amino acids that have similar function to the VEGF-X protein of the invention.

- 5 Also provided by this aspect of the present invention is a nucleic acid molecule such as an antisense molecule capable of hybridising to the nucleic acid molecules according to the invention under high stringency conditions, which conditions would be well
10 known to those skilled in the art.

Stringency of hybridisation as used herein refers to conditions under which polynucleic acids are stable. The stability of hybrids is reflected in the melting
15 temperature (T_m) of the hybrids. T_m can be approximated by the formula:

$$81.5^{\circ}\text{C} + 16.6(\log_{10}[\text{Na}^+]) + 0.41 (\% \text{G}\&\text{C}) - 600/l$$

- 20 wherein l is the length of the hybrids in nucleotides. T_m decreases approximately by 1-1.5°C with every 1% decrease in sequence homology.

The term "stringency" refers to the hybridisation
25 conditions wherein a single-stranded nucleic acid joins with a complementary strand when the purine or pyrimidine bases therein pair with their corresponding base by hydrogen bonding. High stringency conditions favour homologous base pairing whereas low stringency
30 conditions favour non-homologous base pairing.

"Low stringency" conditions comprise, for example, a temperature of about 37°C or less, a formamide concentration of less than about 50%, and a moderate
35 to low salt (SSC) concentration; or, alternatively, a temperature of about 50°C or less, and a moderate to high salt (SSPE) concentration, for example 1M NaCl.

- 5. -

"High stringency" conditions comprise, for example, a temperature of about 42°C or less, a formamide concentration of less than about 20%, and a low salt (SSC) concentration; or, alternatively, a temperature of about 65°C, or less, and a low salt (SSPE) concentration. For example, high stringency conditions comprise hybridization in 0.5 M NaHPO₄, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C (Ausubel, F.M. et al. Current Protocols in Molecular Biology, Vol. I, 1989; Green Inc. New York, at 2.10.3).

"SSC" comprises a hybridization and wash solution. A stock 20X SSC solution contains 3M sodium chloride, 0.3M sodium citrate, pH 7.0.

"SSPE" comprises a hybridization and wash solution. A 1X SSPE solution contains 180 mM NaCl, 9mM Na₂HPO₄, and 1 mM EDTA, pH 7.4.

The nucleic acid capable of hybridising to nucleic acid molecules according to the invention will generally be at least 70%, preferably at least 80 or 90% and more preferably at least 95% homologous to the nucleotide sequences according to the invention.

The antisense molecule capable of hybridising to the nucleic acid according to the invention may be used as a probe or as a medicament or may be included in a pharmaceutical composition with a pharmaceutically acceptable carrier, diluent or excipient therefor.

The term "homologous" describes the relationship between different nucleic acid molecules or amino acid sequences wherein said sequences or molecules are related by partial identity or similarity at one or more blocks or regions within said molecules or

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sequences.

5 The present invention also comprises within its scope proteins or polypeptides encoded by the nucleic acid molecules according to the invention or a functional equivalent, derivative or bioprecursor thereof.

10 Therefore, according to a further aspect of the present invention, there is provided a VEGF-X protein, or a functional equivalent, derivative or bioprecursor thereof, comprising an amino acid sequence from position 23 to 345 of the sequence as illustrated in Figure 10, or alternatively which amino acid sequence comprises the complete sequence of Figure 10. A
15 further aspect of the invention comprises a VEGF-X protein, or a functional equivalent, derivative or bioprecursor thereof, encoded by a nucleic acid molecule according to the invention. Preferably, the VEGF-X protein encoded by said nucleic acid molecule
20 comprises the sequence from position 23 to 345 of the amino acid sequence as illustrated in Figure 10, or which sequence alternatively comprises the sequence of amino acids of Figure 10.

25 The DNA molecules according to the invention may, advantageously, be included in a suitable expression vector to express VEGF-X encoded therefrom in a suitable host. Incorporation of cloned DNA into a suitable expression vector for subsequent
30 transformation of said cell and subsequent selection of the transformed cells is well known to those skilled in the art as provided in Sambrook et al. (1989), molecular cloning, a laboratory manual, Cold Spring Harbour Laboratory Press.

35 An expression vector according to the invention includes a vector having a nucleic acid according to

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the invention operably linked to regulatory sequences, such as promoter regions, that are capable of effecting expression of said DNA fragments. The term "operably linked" refers to a juxta position wherein the components described are in a relationship permitting them to function in their intended manner. Such vectors may be transformed into a suitable host cell to provide for expression of a polypeptide according to the invention. Thus, in a further aspect, the invention provides a process for preparing polypeptides according to the invention which comprises cultivating a host cell, transformed or transfected with an expression vector as described above under conditions to provide for expression by the vector of a coding sequence encoding the polypeptides, and recovering the expressed polypeptides.

The vectors may be, for example, plasmid, virus or phage vectors provided with an origin of replication, and optionally a promoter for the expression of said nucleotide and optionally a regulator of the promoter.

The vectors may contain one or more selectable markers, such as, for example, ampicillin resistance.

Regulatory elements required for expression include promoter sequences to bind RNA polymerase and transcription initiation sequences for ribosome binding. For example, a bacterial expression vector may include a promoter such as the lac promoter and for translation initiation the Shine-Dalgarno sequence and the start codon AUG. Similarly, a eukaryotic expression vector may include a heterologous or homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment of the

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ribosome. Such vectors may be obtained commercially or assembled from the sequences described by methods well known in the art.

5 Nucleic acid molecules according to the invention may be inserted into the vectors described in an antisense orientation in order to provide for the production of antisense RNA. Antisense RNA or other antisense nucleic acids may be produced by synthetic means.

10

In accordance with the present invention, a defined nucleic acid includes not only the identical nucleic acid but also any minor base variations including in particular, substitutions in cases which result in a
15 synonymous codon (a different codon specifying the same amino acid residue) due to the degenerate code in conservative amino acid substitutions. The term "nucleic acid sequence" also includes the
20 complementary sequence to any single stranded sequence given regarding base variations.

The present invention also advantageously provides nucleic acid sequences of at least approximately 10 contiguous nucleotides of a nucleic acid according to
25 the invention and preferably from 10 to 50 nucleotides even more preferably, the nucleic acid sequence comprise the sequences illustrated in Figure 3. These sequences may, advantageously be used as probes or primers to initiate replication, or the like. Such
30 nucleic acid sequences may be produced according to techniques well known in the art, such as by recombinant or synthetic means. They may also be used in diagnostic kits or the like for detecting the presence of a nucleic acid according to the invention.
35 These tests generally comprise contacting the probe with the sample under hybridising conditions and detecting for the presence of any duplex or triplex

formation between the probe and any nucleic acid in the sample.

5 The nucleic acid sequences according to this aspect of the present invention comprise the sequences of nucleotides illustrated in Figures 3 and 5.

10 According to the present invention these probes may be anchored to a solid support. Preferably, they are present on an array so that multiple probes can simultaneously hybridize to a single biological sample. The probes can be spotted onto the array or synthesised *in situ* on the array. (See Lockhart et al., Nature Biotechnology, vol. 14, December 1996
15 "Expression monitoring by hybridisation to high density oligonucleotide arrays". A single array can contain more than 100, 500 or even 1,000 different probes in discrete locations.

20 The nucleic acid sequences, according to the invention may be produced using such recombinant or synthetic means, such as for example using PCR cloning mechanisms which generally involve making a pair of primers, which may be from approximately 10 to 50
25 nucleotides to a region of the gene which is desired to be cloned, bringing the primers into contact with mRNA, cDNA, or genomic DNA from a human cell, performing a polymerase chain reaction under conditions which brings about amplification of the
30 desired region, isolating the amplified region or fragment and recovering the amplified DNA. Generally, such techniques are well known in the art, such as described in Sambrook et al. (Molecular Cloning: a Laboratory Manual, 1989).

35 The nucleic acids or oligonucleotides according to the invention may carry a revealing label. Suitable

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labels include radioisotopes such as ^{32}P or ^{35}S , enzyme labels or other protein labels such as biotin or fluorescent markers. Such labels may be added to the nucleic acids or oligonucleotides of the invention and
5 may be detected using known techniques per se.

Advantageously, human allelic variants or polymorphisms of the DNA molecule according to the invention may be identified by, for example, probing
10 cDNA or genomic libraries from a range of individuals, for example, from different populations. Furthermore, nucleic acids and probes according to the invention may be used to sequence genomic DNA from patients using techniques well known in the art, such as the
15 Sanger Dideoxy chain termination method, which may, advantageously, ascertain any predisposition of a patient to certain disorders associated with a growth factor according to the invention.

20 The protein according to the invention includes all possible amino acid variants encoded by the nucleic acid molecule according to the invention including a polypeptide encoded by said molecule and having conservative amino acid changes. Conservative amino
25 acid substitution refers to a replacement of one or more amino acids in a protein as identified in Table 1. Proteins or polypeptides according to the invention further include variants of such sequences, including naturally occurring allelic variants which are
30 substantially homologous to said proteins or polypeptides. In this context, substantial homology is regarded as a sequence which has at least 70%, preferably 80 or 90% and preferably 95% amino acid
35 homology with the proteins or polypeptides encoded by the nucleic acid molecules according to the invention. The protein according to the invention may be recombinant, synthetic or naturally occurring, but is

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preferably recombinant.

5 The nucleic acid or protein according to the invention may be used as a medicament or in the preparation of a medicament for treating cancer or other diseases or conditions associated with expression of VEGF-X protein.

10 Advantageously, the nucleic acid molecule or the protein according to the invention may be provided in a pharmaceutical composition together with a pharmacologically acceptable carrier, diluent or excipient therefor.

15 The present invention is further directed to inhibiting VEGF-X *in vivo* by the use of antisense technology. Antisense technology can be used to control gene expression through triple-helix formation of antisense DNA or RNA, both of which methods are
20 based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion or the mature DNA sequence, which encodes for the protein of the present invention, is used to design an antisense RNA oligonucleotide of from 10 to 50 base pairs in length.
25 A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription (triple-helix - see Lee et al. Nucl. Acids Res., 6:3073 (1979); Cooney et al., Science, 241:456 (1988); and Dervan et al., Science, 251: 1360 (1991), thereby
30 preventing transcription and the production of VEGF-X. The antisense RNA oligonucleotide hybridises to the mRNA *in vivo* and blocks translation of an mRNA molecule into the VEGF-X protein (antisense - Okano, J. Neurochem., 56:560 (1991); Oligodeoxynucleotides as
35 Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)).

Alternatively, the oligonucleotide described above can be delivered to cells by procedures in the art such that the anti-sense RNA and DNA may be expressed in vivo to inhibit production of VEGF-X in the manner described above.

Antisense constructs to VEGF-X, therefore, may inhibit the angiogenic activity of VEGF-X and prevent the further growth of or even regress solid tumours, since angiogenesis and neovascularization are essential steps in solid tumour growth. These antisense constructs may also be used to treat rheumatoid arthritis, psoriasis and diabetic retinopathy which are all characterized by abnormal angiogenesis.

A further aspect of the invention provides a host cell or organism, transformed or transfected with an expression vector according to the invention. The host cell or organism may advantageously be used in a method of producing VEGF-X, which comprises recovering any expressed VEGF-X from the host or organism transformed or transfected with the expression vector.

According to a further aspect of the invention there is also provided a transgenic cell, tissue or organism comprising a transgene capable of expressing VEGF-X protein according to the invention. The term "transgene capable of expression" as used herein means a suitable nucleic acid sequence which leads to expression of VEGF-X or proteins having the same function and/or activity. The transgene, may include, for example, genomic nucleic acid isolated from human cells or synthetic nucleic acid, including DNA integrated into the genome or in an extrachromosomal state. Preferably, the transgene comprises the nucleic acid sequence encoding the proteins according to the invention as described herein, or a functional

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- fragment of said nucleic acid. A functional fragment of said nucleic acid should be taken to mean a fragment of the gene comprising said nucleic acid coding for the proteins according to the invention or
- 5 a functional equivalent, derivative or a non-functional derivative such as a dominant negative mutant, or bioprecursor of said proteins. For example, it would be readily apparent to persons skilled in the art that nucleotide substitutions or
- 10 deletions may be used using routine techniques, which do not affect the protein sequence encoded by said nucleic acid, or which encode a functional protein according to the invention.
- 15 VEGF-X protein expressed by said transgenic cell, tissue or organism or a functional equivalent or bioprecursor of said protein also forms part of the present invention.
- 20 Antibodies to the protein or polypeptide of the present invention may, advantageously, be prepared by techniques which are known in the art. For example, polyclonal antibodies may be prepared by inoculating a host animal, such as a mouse or rabbit, with the
- 25 polypeptide according to the invention or an epitope thereof and recovering immune serum. Monoclonal antibodies may be prepared according to known techniques such as described by Kohler R. and Milstein C., Nature (1975) 256, 495-497. Advantageously, such
- 30 antibodies may be included in a kit for identifying VEGF-X in a sample, together with means for contacting the antibody with the sample.
- 35 Advantageously, the antibody according to the invention may also be used as a medicament or in the preparation of a medicament for treating tumours or other diseases associated with expression of VEGF-X.

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The invention also further provides a pharmaceutical composition comprising said antibody together with a pharmaceutically acceptable carrier diluent or excipient therefor.

5

Proteins which interact with the polypeptide of the invention may be identified by investigating protein-interactions using the two-hybrid vector system first proposed by Chien et al., (1991) Proc. Natl. Acad. Sci. USA 88 : 9578-9582.

10

This technique is based on functional reconstitution in vivo of a transcription factor which activates a reporter gene. More particularly the technique comprises providing an appropriate host cell with a DNA construct comprising a reporter gene under the control of a promoter regulated by a transcription factor having a DNA binding domain and an activating domain, expressing in the host cell a first hybrid DNA sequence encoding a first fusion of a fragment or all of a nucleic acid sequence according to the invention and either said DNA binding domain or said activating domain of the transcription factor, expressing in the host at least one second hybrid DNA sequence, such as

20 a library or the like, encoding putative binding proteins to be investigated together with the DNA binding or activating domain of the transcription factor which is not incorporated in the first fusion; detecting any binding of the proteins to be

25 investigated with a protein according to the invention by detecting for the presence of any reporter gene product in the host cell; optionally isolating second hybrid DNA sequences encoding the binding protein.

30

35

An example of such a technique utilises the GAL4 protein in yeast. GAL4 is a transcriptional activator of galactose metabolism in yeast and has a separate

domain for binding to activators upstream of the galactose metabolising genes as well as a protein binding domain. Nucleotide vectors may be constructed, one of which comprises the nucleotide
5 residues encoding the DNA binding domain of GAL4. These binding domain residues may be fused to a known protein encoding sequence, such as for example, the nucleic acids according to the invention. The other vector comprises the residues encoding the protein
10 binding domain of GAL4. These residues are fused to residues encoding a test protein. Any interaction between polypeptides encoded by the nucleic acid according to the invention and the protein to be tested leads to transcriptional activation of a
15 reporter molecule in a GAL-4 transcription deficient yeast cell into which the vectors have been transformed. Preferably, a reporter molecule such as β -galactosidase is activated upon restoration of transcription of the yeast galactose metabolism genes.

20 A further aspect of the present invention also provides a method of identifying VEGF-X in a sample, which method comprises contacting said sample with an antibody according to the invention and monitoring for
25 any binding of any proteins to said antibody. A kit for identifying the presence of VEGF-X in a sample is also provided comprising an antibody according to the invention and means for contacting said antibody with said sample.

30 VEGF-X may be recovered and purified from recombinant cell cultures by methods known in the art, including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography,
35 phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxyapatite chromatography and lectin

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chromatography.

5 The VEGF-X protein of the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the
10 polypeptides of the present invention may be glycosylated with mammalian or other eukaryotic carbohydrates or may be non-glycosylated.

15 VEGF-X is particularly advantageous as a wound healing agent, where, for example, it is necessary to re-vascularize damaged tissues, or where new capillary angiogenesis is important. Accordingly, VEGF-X may be used for treatment of various types of wounds such as for example, dermal ulcers, including pressure sores, venous ulcers, and diabetic ulcers. In addition, it
20 can be used in the treatment of full-thickness burns and injuries where angiogenesis is desired to prepare the burn in injured sites for a skin graft and flap. In this case, VEGF-X or the nucleic acid encoding it
25 may be applied directly to the wound. VEGF-X may be used in plastic surgery when reconstruction is required following a burn, other trauma, or even for cosmetic purposes.

30 An important application of VEGF-X is to induce the growth of damaged bone, periodontium or ligament tissue. For example, it may be used in periodontal disease where VEGF-X is applied to the roots of the diseased teeth, leading to the formation of new bone
35 and cementum with collagen fibre ingrowths. It can be used for regenerating supporting tissues of teeth, including alveolar bone, cementum and periodontal

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ligament, that have been damaged by disease and trauma.

5 Since angiogenesis is important in keeping wounds clean and non-infected, VEGF-X may be used in association with surgery and following the repair of cuts. It should be particularly useful in the treatment of abdominal wounds where there is a high risk of infection.

10 VEGF-X can also be used for the promotion of endothelialization in vascular graft surgery. In the case of vascular grafts using either transplanted or synthetic material, VEGF-X may be applied to the surface of the graft or at the junction to promote the growth of the vascular endothelial cells. One derivation of this is that VEGF-X can be used to repair the damage of myocardial and other occasions where coronary bypass surgery is needed by stimulating the growth of the transplanted tissue. Related to this is the use of VEGFX to repair the cardiac vascular system after ischemia.

25 The protein of the present invention may also be employed in accordance with the present invention by expression of such protein *in vivo*, which is often referred to as "gene therapy".

30 Thus, for example, cells such as bone marrow cells may be engineered with a polynucleotide (DNA or RNA) encoding for the protein *ex vivo* as defined herein, the engineered cells are then provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, cells may be engineered by procedures known in the art by use of a retroviral particle containing RNA encoding for the protein of the present invention.

Similarly, cells may be engineered *in vivo* for expression of the protein *in vivo*, for example, by procedures known in the art.

- 5 A further aspect of the invention comprises a method of treating a disorder mediated by expression of a protein according to the invention, by administering to a patient an amount of an antisense molecule as described herein, in sufficient concentration to
10 alleviate or reduce the symptoms of said disorder.

- Compounds which inhibit or enhance angiogenesis may be identified by providing a host cell or organism according to the invention or a transgenic cell,
15 tissue or organism according to the invention, contacting a test compound with said cell, tissue or organism and monitoring for the effect of said compound compared to a cell tissue or organism which has not been contacted with said compound. These
20 compounds may themselves be used as a medicament or included in a pharmaceutical composition for treatment of disorders mediated by inappropriate vascularisation or angiogenic activity.

- 25 The present inventors have also, advantageously, identified in the sequence encoding the VEGF-X protein a CUB domain, which has heretofore not previously been identified in VEGF-type growth factors. The VEGF-X protein may therefore exert dual regulatory effects
30 via interaction with the VEGF tyrosine kinase receptors or with neuropilin receptors mediated by the CUB domain. Thus, the sequence encoding said CUB domain may be included in an expression vector for subsequent transformation of a host cell, tissue or
35 organism.

VEGF-X or fragments thereof may be able to modulate

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the effects of pro-angiogenic growth factors such as VEGF as indicated in the findings presented in the examples below that the N-terminal part of the VEGF-X protein, a CUB-like domain, is able to inhibit VEGF-stimulated proliferation of HUVECs. VEGF-X or fragments thereof may therefore be useful in therapy of conditions involving inappropriate angiogenesis. Inhibition of the angiogenic activity of VEGF has been linked with inhibition of tumour growth in several models eg Kim K. J. et al, Nature 362:841-844, (1993). Additionally, agents able to inhibit angiogenesis would be expected to be useful in treating other angiogenesis-dependent diseases such a retinopathy, osteoarthritis and psoriasis (Folkman, J., Nature Medicine 1:27-31, (1995)).

As identified in more detail in the Examples described herein the present inventors have surprisingly identified that the CUB domain of VEGF-X is able to inhibit stimulation of proliferation of HUVECs induced by either VEGF or bFGF. The CUB domain may, therefore, be utilised as a therapeutic agent for inhibition of angiogenesis and for treatment of condition associated with inappropriate vascularisation or angiogenesis.

Therefore according to a further aspect of the invention there is provided a method of inhibiting angiogenic activity and inappropriate vascularisation including formation and proliferation of new blood vessels, growth and development of tissues, tissue regeneration and organ and tissue repair in a subject said method comprising administering to said subject an amount of a polypeptide having an amino acid sequence from position 40 to 150 of the sequence illustrated in Figure 10 or a nucleic acid molecule encoding the CUB domain according to the invention in

sufficient concentration to reduce or prevent said angiogenic activity.

5 Furthermore there is also provided a method of treating or preventing any of cancer, rheumatoid arthritis, psoriasis and diabetic retinopathy, said method comprising administering to said subject an amount of a polypeptide having an amino acid sequence from position 40 to 150 of the sequence illustrated
10 in Figure 10 or a nucleic acid molecule encoding the CUB domain according to the invention in sufficient concentration to treat or prevent said disorders.

15 The CUB domain may also be used to identify compounds that inhibit or enhance angiogenic activity such as inappropriate vascularisation, in a method comprising contacting a cell expressing a VEGF receptor and/or a neuropilin 1 or 2 type receptor with said compound in the presence of a VEGF-X protein according to the
20 invention and monitoring for the effect of said compound or said cell when compared to a cell which has not been contacted with said compound. Such compounds may then be used as appropriate to prevent or inhibit angiogenic activity to treat the disorders
25 or conditions described herein, or in a pharmaceutical composition. An antibody to said CUB domain may also be useful in identifying other proteins having said sequences.

30

35

Deposited Plasmids

		<u>Date of Deposit</u>	<u>Accession No.</u>
	Plasmid VEGFX/PCR2.1		
5	1TOPO FL	1 March 1999	LMBP 3925
	Plasmid VEGFX/pRSETB BD		
	amino acids	1 March 1999	LMBP 3926
10	G230-G345		
	Plasmid VEGFX/PCR.2.1		
	FL Clone 9	20 October 1999	LMBP 3977
15	Plasmid VEGF-X CUB		
	PET22b	20 December 1999	-----

20 The above plasmids were deposited at the Belgian Coordinated Collections of Microorganisms (BCCM) at Laboratorium Voor Moleculaire Biologie-Plasmidencollectie (LMBP) B-9000, Ghent, Belgium, in accordance with the provisions of the Budapest Treaty of 28 April 1977.

25 The invention may be more clearly understood with reference to the accompanying example, which is purely exemplary, with reference to the accompanying drawings, wherein:

30 Figure 1: is a DNA sequence identified in the Incyte LifeSeq™ database coding for a novel VEGF-X protein.

35 Figure 2: is an illustration of amino acid sequence of the nucleic acid sequence of Figure 1.

- Figure 3: is an illustration of PCR primer sequences utilised to identify the VEGF-X protein according to the invention.
- 5
Figure 4: is a diagrammatic illustration of the spatial relationships in the VEGF-X sequence of the clones identified using the PCR primer sequences of Figure 3.
- 10
Figure 5: is an illustration of the nucleotide sequences of the 5' RACE primers used to identify the 5' end of the VEGF-X open reading frame.
- 15
Figure 6: is an illustration of the sequence obtained from the RACE experiment.
- 20
Figure 7: is an illustration of the nucleotide sequences obtained from the search of LifeSeqTM database using the sequence in Figure 6.
- 25
Figure 8: is an illustration of the primers used to clone the entire coding sequence of VEGF-X.
- 30
Figure 9: is an illustration of the entire coding sequence of VEGF-X.
- 35
Figure 10: is an illustration of the predicted amino acid sequence of the nucleotide sequence of Figure 9.
- Figure 11: is an alignment of the sequence of

Figure 10 with the sequences of VEGF-A to D.

Figure 12: is an illustration of variant sequences of the VEGF-X protein according to the invention.

Figure 13: is an illustration of the oligonucleotide primers used for E.coli expression of VEGF-X domains and for expression of the full length sequence of VEGF-X in a baculovirus/insect cell expression system.

15 Figure 14: depicts nucleic acid sequences of 18
 human EST clones obtained from a BLAST
 search of the LifeSeq™ database used
 to identify the full sequence encoding
20 VEGF-X.

Figure 15: depicts the nucleotide sequences of 50 human EST clones obtained from the LifeSeq™ database.

25

Figure 16: is an illustration of nucleotide sequences utilised as primers to identify the nucleotide sequence encoding VEGF-X.

30 Figure 17: is a nucleotide sequence coding for a partial VEGF-X protein according to the invention.

35 Figure 18: is an illustration of a partial
nucleotide sequence encoding VEGF-X
protein according to the invention.

- Figure 19: is an illustration of a DNA and polypeptide sequence used for mammalian cell expression of VEGF-X. The predicted VEGF-X signal sequence is in lower case letters. The C-terminal V5 epitope and His6 sequences are underlined.
- Figure 20: is an illustration of a DNA and polypeptide sequence used for baculovirus/insect cell expression of VEGF-X. In the polypeptide sequence the signal sequence is shown in lower case. The N-terminal peptide tag added to the predicted mature VEGF-X sequence is underlined.
- Figure 21: is an illustration of a DNA and polypeptide sequence used for *E. coli* expression of VEGF-X. The polypeptide sequences at the N- and C- termini derived from the MBP fusion and His6 tag respectively are underlined.
- Figure 22: illustrates the disulphide-linked dimerisation of VEGF-X. Protein samples were analysed by SDS-PAGE. Prior to loading the gel, samples were heated to 95°C for 5 minutes in sample buffer in the presence (+) or absence (-) of reducing agent. (A) samples from COS cell expression of a C-terminally V5/His6 peptide-tagged construct. The left hand panel is total conditioned medium, the right hand panel is material purified on Nickel agarose resin. Reduced monomer

- 25 -

5 and putative disulphide-linked, non-reduced dimer are indicated by arrows. There appears to be proteolysis of the protein during purification. Gels were
10 blotted onto nylon membranes and protein detected with an anti V5 monoclonal antibody. (B) Samples from *E. coli* expression of a maltose-binding protein/His6 dual fusion construct. M indicates the molecular weight markers (Benchmark, LifeTechnologies). The gel was stained with Coomassie Blue by
15 standard procedures. The fusion protein has an apparent molecular weight of 80kDa.

Figure 23: illustrates the glycosylation of VEGF-X. VEGF-X was purified from the culture supernatant of COS cells transfected with the pcDNA6/V5-His construct. Supernatants were
20 harvested 72h post-transfection and purified on nickel resin. Samples were then treated with EndoH (+) or untreated (-) before SDS-PAGE and blotting, as described in the legend to Figure 22.

Figure 24: is an illustration of the DNA and polypeptide sequence used for *E. coli* expression of the VEGF-like domain of VEGF-X. Polypeptide sequences at the N-terminus of the protein derived from the vector are underlined.
30

Figure 25: shows expression of the VEGF-X VEGF domain in *E. coli*. Lane 1-10µl broad
35

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range marker (New England Biolabs),
lane 2-10 μ l unreduced sample, lane 3-
10 μ l reduced sample. The reduced PDGF
domain protein (lane 3) has an
apparent molecular weight of
approximately 19kDa on SDS-PAGE.

Figure 26: illustrates a DNA and polypeptide
sequence used for *E. coli* expression
of the CUB-like domain of VEGF-X. The
polypeptide sequence at the N-terminus
derived from the vector-encoded signal
and the introduced His6 tag are
underlined.

Figure 27: shows expression of the VEGF-X CUB
domain in *E. coli*. The CUB domain
protein was purified on Nickel chelate
resin. The protein migrates at
approximately 23kDa on SDS-PAGE.

Figure 28: illustrates the effect of truncated
VEGF-X (CUB domain) on HUVEC
proliferation. (A) Human Umbilical
Vein Endothelial Cells (one-day-
treatment). (B) Human Umbilical Vein
Endothelial Cells (24-hour starving
followed by one-day-treatment). (C)
Effect of VEGF-A₁₆₅ and VEGF-X CUB
domain on the proliferation of HUVEC
(two-day-treatment).

Figure 29: depicts the tissue distribution of
VEGF-X mRNA analysed by Northern
blotting and RT-PCR in (A) normal
tissues and (B) tumour tissue and cell
lines.

Figure 30: depicts the partial intron/exon structure of the VEGF-X gene. (A) Genomic DNA sequences of 2 exons determined by sequencing; exon sequence is in upper case, intron sequence is in lower case. (B) Shows the location of splice sites within the VEGF-X cDNA sequence. The location of mRNA splicing events is indicated by vertical lines. The cryptic splice donor/acceptor site at nt. 998/999 (diagonal lines) gives rise to the splice variant forms of VEGF-X. No splice site information is given for the region shown in italics.

Figure 31: is a graphic representation of the effect of FL-VEGF-X on HuVEC proliferation: (24 hour serum starvation followed by one day treatment).

Figure 32: is a graphic representation of the combined effect of truncated VEGF-X (CUB domain) and human recombinant VEGF₁₆₅ on HuVEC proliferation: (24 hour serum starvation followed by two day treatment).

Figure 33: is a graphic representation of the combined effect of the CUB domain and human recombinant bFGF on HuVEC proliferation: (24 hour serum starvation followed by two day treatment).

Figure 34: is a graphic representation of the

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results of a LDH assay for testing cytotoxicity of the CUB domain or the CUB domain with rhVEGF₁₆₅.

5 Figure 35: is a graphic representation of the results obtained from a LDH assay for testing cytotoxicity of the CUB domain or CUB domain with rh-bFGF.

10 A BLAST (Basic Local Alignment Search Tool; Altschul et al., 1990 J. Mol. Biol. 215, 403-410) search was performed in the proprietary LifeSeq™ human EST database (Incyte Pharmaceuticals, Inc., Palo Alto, CA, USA). BLAST produces alignments of both
15 nucleotide and amino acid sequences to determine sequence similarity. Because of the local nature of the alignments, BLAST is especially useful in determining exact matches or in identifying homologues. While it is useful for matches which do
20 not contain gaps, it is inappropriate for performing motif-style searching. The fundamental unit of BLAST algorithm output is the High-scoring Segment Pair (HSP).

25 Eighteen human EST clones (Figure 14) with high similarity to the previously identified VEGF proteins were identified and a further fifty EST clones (Figure 15) were identified using these sequences as query sequences, allowing us to deduce the putative
30 sequence for the new VEGF-X protein. The sequences obtained were compared to known sequences to determine regions of homology and to identify the sequence as a novel VEGF-type protein. Using the DNA sequence information in the databases we were able to
35 prepare suitable primers having the sequences of VEGF-X 1-10 illustrated in Figure 3 for use in subsequent RACE experiments to obtain the complete

DNA sequence for the VEGF-X gene.

Cloning

5 A profile was developed based on the VEGF-like domain,
in existing VEGF sequences (VEGF-A, B, C and D).
This was used to search the public databases and the
Incyte LifeSeq™ database. No significant novel
10 matching sequences were found in the public
databases. All of the matching sequences found in
the LifeSeq™ database (~1000) were assembled to give
a smaller number of sequences (~30), which included
the known VEGFs and a potential novel VEGF (figures
1 and 2). This sequence was named VEGF-X.
15

Oligonucleotides were designed to amplify the VEGF-X
sequence from cDNA (figure 3). The ESTs found in
LifeSeq™ were from a range of tissues, with a slight
predominance of sequences from ovary, testis,
20 placenta and lung (Figure 14 and 15). Accordingly
the oligonucleotides were used to amplify cDNA
derived from lung and placenta. First-round PCR
products were found at ~200bp larger than the
expected sizes, while 3 major species appeared after
25 a second round of PCR amplification, the smallest of
which was of the expected size. These fragments were
cloned and sequenced. The smallest fragment did
indeed have the sequence originally identified from
the LifeSeq database, while the others contained
30 insertions (figure 4).

As the first round of amplification suggested that
the major species found in cDNA from ovary and
placenta was not that originally identified in the
LifeSeq™ database, the focus of effort was switched
35 to the presumed major species (it seemed likely that

- 30 -

clones 57, 25-27 and 2.1kb clones 1-3 in fig 4 represented the major mRNA species). Conceptual translation of the DNA sequences of these cloned PCR fragments indicated that the complete open reading frame was not present in the clones or in the sequence from LifeSeq™. While all clones contained the same sequence in the region of the translation termination codon, indicating that the end of the open reading frame had been identified, the 5' end of the open reading frame had not been cloned. 5' RACE experiments were therefore carried out in order to find the start of the reading frame. PCR primers designed for RACE experiments are shown in figure 5. RACE PCR products were sequenced directly. Sequence could be obtained from the 3' end of these RACE products but not from the 5' end; probably because the products were not cloned and were therefore heterogeneous at the 5' end. This new sequence was assembled with the existing cloned sequence to give the sequence shown in figure 6. Searching the LifeSeq™ database with this sequence identifies ESTs which extend the sequence a further 140bp in the 5' direction and a further 160bp in the 3' direction (figure 7). This longer contig was used to design oligonucleotide primers to amplify the entire coding sequence (these primer sequences are shown in figure 8). PCR was carried out using primers 5'-1 and vegfX10 (in order to clone a "full-length" cDNA), and with primers 5'-1 and vegfX6 (in order to clone the full coding region, see figure 3 for sequences of vegfX10 and vegfX6). A number of clones were obtained for the shorter fragment, of which clones 4 and 7 contain no PCR errors (sequence of clones 4 & 7 in figure 9). A single clone was obtained for the longer fragment (clone 9), but this sequence appears to contain 2 PCR errors.

The predicted polypeptide from these longer contigs is shown in figure 10. Amino acids 1-22 are predicted to encode a signal sequence (von Heijne, 1986, *Nucleic Acids Res.* 14, 4683-4690). Figure 11 shows an alignment of the protein sequence with VEGFs A-D. The region homologous to the other VEGFs is located towards the C-terminus of the protein. As the VEGF homology domain is expected to belong to the TGF-beta superfamily of growth factors and to consist of a dimer containing both intra- and intermolecular disulphide bonds, initial alignments focussed on the cysteines. However, mapping of the sequence onto the known x-ray structure of the VEGF-A receptor-binding domain (Muller et al (1997) *Proc. Natl. Acad. Sci USA* 94, 7192-7197) suggests that the alignment in figure 11 is plausible, as the extra 4 cysteine residues within the VEGF-homology region of VEGF-X (compared to this region of VEGF-A) correspond to residues which are spatially close in VEGF-A, and may therefore be able to form disulphide bonds.

A search of the PFAM database of protein domains with the full-length polypeptide sequence from figure 10 identifies two domain consensus sequences within the polypeptide. The more C-terminal domain is a "VEGF" domain: (the known VEGFs all contain this domain and the structure of this region of VEGF-A is similar to that of PDGF). Additionally towards the N-terminus of the polypeptide there is a CUB domain (amino acids ~40-150). The CUB domain is a 100-110 amino acid extracellular domain found in a number of developmentally-regulated proteins. When the full-length protein is used to search the protein databases using the BLAST 2 algorithm, the scores for matches to CUB domain-containing proteins are more

significant than those to the other VEGFs.

Interestingly, the most significant matches are to the CUB domains of Neuropilins, and Neuropilin-1 was recently identified as a receptor of one of the VEGF-A isoforms VEGF-A₁₆₅ (Soker et al. (1998) *Cell* 92, 735-745).

Assuming that the variant sequences isolated by PCR (i.e. the smaller PCR fragments) use the same translation initiation site as the full-length sequence, they would result in production of the variant proteins shown in figure 12. It may be significant that both of these variant proteins retain the CUB domain and delete all or part of the VEGF-like domain. The production of these variant sequences can be explained by the use of a cryptic splice donor/acceptor site within the VEGF-X sequence (figure 30B, between nt. 998/999): one variant arises by splicing out of the region between nt. 729-998, the other by splicing out of the region between nt. 999-1187.

Expression

25 Full-length expression constructs

Mammalian cells

Clone 4 containing the full CDS of VEGF-X (see figure 9), was used to generate constructs for expression of full-length protein. The sequence was amplified by PCR and cloned into the vector pCDNA6/V5-His so as to add a C-terminal V5 epitope tag and His₆ tag. The DNA and polypeptide sequence in this vector is shown in figure 19. Transient expression in COS cells followed by western blotting and detection via an anti-V5 mAb demonstrates the secretion of a protein of ~50K into the medium in transfected cells only

(figure 22A). This construct can also be used to generate VEGF-X expressing stable CHO cell lines.

Baculovirus/Insect-cell expression system

5 For expression in the baculovirus/insect cell system the DNA encoding the predicted mature VEGF-X polypeptide sequence was fused to a sequence encoding a signal derived from melittin, a secreted insect protein. An N-terminal 6His tag was also added to
10 facilitate purification. The insert was then cloned into the baculovirus expression vector pFASTBAC. The DNA and polypeptide sequence of this construct is shown in figure 20. Infection of *Trichoplusia ni* Hi5 cells with this recombinant baculovirus results in
15 the secretion of a protein of approximately 45K into the medium (data not shown).

E.coli

20 The coding region of VEGF-X has been cloned in a variety of ways for expression as a secreted protein in *E.coli*. A particularly useful expression clone carries an N-terminal fusion to the *E.coli* maltose-binding protein (MBP- derived from the expression vector pMAL-p2, New England Biolabs) and a
25 C-terminal fusion to a 6His tag. The DNA and polypeptide sequence of this vector is shown in figure 21. Sequential purification of cell fractions on Ni-NTA resin and amylose resin allows the isolation of the expressed protein (see figure 22B).
30

Expression of fragments

VEGF

35 The VEGF domain of VEGF-X has been expressed in *E.coli*. Similar domains from VEGF-A (Christinger et al. (1996) *PROTEINS: Structure, Function and Genetics* 26, 353-357), and VEGF-D (Achen et al (1998) *Proc.*

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Natl. Acad. Sci USA 95, 548-553) have been shown to be capable of binding to the respective receptors. Expression of these domains was carried out using the bacterium *E.coli*. Additionally, the full-length protein was expressed using the baculovirus/insect cell expression system. The oligonucleotide primers which have been obtained for these experiments are shown in figure 13. The construct directed expression in the bacterial cytoplasm, and as expected the protein was produced in insoluble form in inclusion bodies (the DNA and polypeptide sequence used for PDGF domain expression is shown in figure 24). Inclusion bodies were washed, solubilized with urea and the protein purified under denaturing conditions, before refolding by dialysis to remove the urea. Soluble protein was obtained, but shows little evidence of the disulphide bond linked dimers seen with material derived from animal cells (figure 25, compare with figure 22A & B). It is not clear therefore whether this protein is correctly folded.

CUB

The CUB domain has been expressed as a soluble secreted protein in *E.coli* (figure 26). The protein was purified by binding to Ni-NTA resin (figure 27) and assayed for activity on HUVECs in an in-vitro proliferation assay.

Properties of the VEGF-X protein

The transient mammalian cell expression system described above has been used to generate full-length VEGF-X protein, as shown by antibody detection following Western blotting (see figure 22A).

Disulphide bond linked dimers

The other members of the PDGF family of growth

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factors, the PDGFs and VEGFs, all exist as dimers in which two monomers constituting the dimer are linked by interchain disulphide bonds. The x-ray structures of PDGF-BB (Oefner et al, 1992), and VEGF-A (Müller et al, 1997) are known and indicate that at least these two members of the family contain two interchain disulphide bonds. Practically this means that in SDS-PAGE analysis of these growth factors the presence of interchain disulphide bonds is shown by a large decrease in mobility in the absence of reducing agent (ie. the nonreduced dimer migrates more slowly through the gel than the reduced monomer). This effect was also expected for VEGF-X, and has been demonstrated for the material obtained from transient mammalian cell expression (figure 22A). In the case of the full length material produced in *E.coli* only some 10% of the total VEGF-X protein appears to be present as disulphide bond-linked dimers (figure 22B). However, these results provide evidence that the mammalian cell-derived protein is correctly folded, and that a portion of the *E.coli*-derived protein is too.

Glycosylation

There are 3 predicted potential N-linked glycosylation sites within the VEGF-X protein: at residues 25, 55 and 254 of the polypeptide sequence. The predicted molecular mass of the mature VEGF-X protein is 40kDa, but SDS-PAGE and western blotting (detection via an introduced C-terminal epitope tag-see figure 19) of the full-length protein expressed in COS cells gives a band slightly larger than the expected size (45-50kDa) as well as one at 25kDa (figure 22A). This smaller band is presumed to be a C-terminal proteolysis fragment derived from the full-length molecule (controls from uninfected cells do not show this band), probably corresponding to a

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cleavage between the CUB and VEGF domains. EndoH treatment of the preparation gives a slight mobility change for the full-length protein (figure 23), but for the smaller VEGF domain fragment there is a clear change, indicating that the predicted glycosylation site within the VEGF domain at residue 254 is indeed glycosylated.

Activity of proteins in cell-based assays

Protein samples were tested for activity in cell proliferation, cell migration and *in-vitro* angiogenesis assays. Active samples can also be tested in the *in vivo* matrigel mouse model of angiogenesis.

15

Full-length VEGF-X protein

Conditioned medium derived from COS cells transiently expressing VEGF-X (see figure 22A) displayed no detectable activity in any of the assays. However, as VEGF-X protein could only be detected in this preparation by Western blotting, and not by Coomassie-staining of gels, it is clearly present at very low levels and this may be the reason for the observed lack of activity in the cell proliferation, migration or *in vitro* angiogenesis tests.

25

VEGF domain

The VEGF domain protein described above has been tested in cell proliferation (on a range of cell types), cell migration and *in vitro* angiogenesis assays and has failed to show activity in any of these tests. As suggested above, this may be due to incorrect folding of this protein.

30

CUB domain

The CUB domain protein at the highest dose tested

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(1pg/ml) appears to inhibit proliferation of HUVECs in the absence of other stimulation (figure 28A & B). This effect is also seen following stimulation with the lowest VEGF-A₁₆₅ dose tested (1ng/ml- figure 28C).
5 The CUB domain of VEGF-X therefore appears to show antiproliferative activity on HUVECs, even in the presence of low VEGF-A₁₆₅ doses.

Tissue distribution of mRNA

10 VEGF-A mRNA expression has been shown to be upregulated in a wide variety of human tumors (lung, breast, ovarian, colon, stomach, liver, pancreas, kidney, bladder and prostate- Takahashi et al, 1995). Tumor VEGF-A expression has been shown to correlate
15 with tumor growth rate, microvascular density and tumor metastasis (Takahashi et al, 1995). It was thus of interest to examine the mRNA expression patterns of VEGF-X. Accordingly, Northern blot analysis of mRNA derived from different tissues has
20 been carried out. The results indicate that although the VEGF-X mRNA is expressed at low levels, it is present in a wide range of tissues. PCR amplification of cDNA from a range of tissue sources supports this idea (figure 29A). The major mRNA
25 species is approximately 3.1kb in size. There is no significant upregulation seen in tumour cell lines or in tumour tissues tested (figure 29B), with the possible exception of the cell lines GI-117 (lung carcinoma) and SaOS-2 (osteosarcoma). The results of
30 these initial tissue distribution studies do not, therefore, provide evidence for upregulation of VEGF-X in tumour growth, as is seen with VEGF-A.

Genomic structure of the VEGF-X gene

35 A genomic BAC clone covering the 3' part of the VEGF-X locus was isolated by hybridisation screening

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of nylon filters containing a human BAC library. Direct sequencing of this clone using oligonucleotide primers based on the VEGF-X cDNA sequence allowed the determination of several intron/exon boundaries (figure 30). Interestingly, the position of the mRNA splice site within the PDGF domain (nt 1187/1188 in figure 30B) is conserved with respect to those in the VEGF-A and VEGF-D genes (Tischer et al, 1991; Rocchigiani et al, 1998).

Materials & Methods

PCR, Cloning, DNA sequence determination and BAC screening.

All primers were purchased from Eurogentec, Seraing, Belgium. Insert-specific sequencing primers (15- and 16-mers) were designed by visual inspection of the DNA sequences. DNA was prepared on Qiagen-tip-20 columns or on Qiaquick spin columns (Qiagen GmbH, Düsseldorf, Germany) and recovered from the spin columns in 30µl Tris/EDTA-buffer (10mM TrisHCl pH 7.5, 1 mM EDTA (sodium salt)). Sequencing reactions were performed using BigDye™ Terminator Cycle Sequencing Ready Reaction kits (Perkin Elmer, ABI Division, Foster City, CA, USA) and were run on an Applied Biosystems 377 DNA sequencer (Perkin Elmer, ABI Division, Foster City, CA, USA). Polymerase chain reactions were carried out according to standard procedures (Ausubel et al, 1997). The PCR fragments were cloned into vectors pCR2.1 (Invitrogen, Carlsbad, CA, USA) or pCR-TOPO (Invitrogen, NL) according to the manufacturer's instructions. One of those vectors, plasmid VEGFX/pCR2.1 1TOPO FL was deposited on 1 March 1999 under Accession No. LMBP 3925. After sequence determination, the inserts were cloned into the desired expression vectors (see

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figures 19, 20, 21, 24 & 26).

5 A human genomic BAC library (Genome Systems, Inc., St
Louis, MI, USA) was screened by hybridisation to
oligonucleotides derived from the VEGF-X cDNA
sequence, according to the manufacturer's
instructions. BAC DNA was prepared using a Qiagen
plasmid midi kit (Qiagen GmbH, Düsseldorf, Germany)
10 according to the manufacturer's instructions with
some modifications (after clearing of the lysate from
chromosomal DNA, supernatants from individual
preparations were pooled on a single column (tip
100), and after the 70 % EtOH wash, the pellet was
15 resuspended overnight at 4°C in 100 µl TE). 20-mer
sequencing primers were designed based on the known
cDNA sequence, and sequencing carried out as above.

5' RACE

20 In order to extend the cDNA clone in a 5' direction
RACE reactions were carried out. Since it was known
that the mRNA is present in placenta and skeletal
muscle, Marathon-Ready™ placenta and skeletal muscle
cDNAs were purchased from Clontech (Palo Alto CA.
25 USA) and used according to the manufacturer's
instructions. DNA fragments were excised from
agarose gels, purified using QiaQuick PCR
purification columns (Qiagen GmbH, Düsseldorf,
Germany) and sequenced directly.

30

VEGF-X protein expression and purification
DNA fragments encoding the desired protein sequences
were amplified by PCR and cloned into appropriate
expression vector systems.

35

For mammalian cell expression, the full coding

- 40 -

sequence was cloned into the vector pCDNA6/V5-his (Invitrogen Leek, NL, see figure 19 for construct sequence), so as to add a C-terminal peptide tag to assist in detection and purification.

5

For insect cell expression the sequence of the predicted mature polypeptide was initially amplified to add an N-terminal 6His peptide and then cloned into the pMelBacB vector (Invitrogen, Leek, NL) to add an insect cell signal sequence. The entire insert was then PCR-cloned into the vector pFASTBAC-1 (LifeTechnologies, Gaithersburg, MA, USA) for construction of a baculovirus according to the manufacturer's instructions.

15

For *E.coli* expression, the coding region was PCR amplified to add a C-terminal 6His tag and then cloned into the vector pMAL-p2 (New England Biolabs, Beverly, MA, USA). The coding sequence of this construct is shown in figure 21). The protein was purified first on Ni-NTA resin (Qiagen GmbH, Düsseldorf, Germany) and then on amylose resin (New England Biolabs, Beverly, MA, USA), according to the manufacturers' instructions.

25

DNA sequences encoding the CUB and VEGF domain fragments of VEGF-X were PCR amplified and cloned into pET22b and pET21a (Novagen, Madison, WI, USA) respectively. The CUB domain protein was prepared either from the periplasm or medium of induced cultures by standard methods (Ausubel et al, 1997). The protein was initially purified by precipitation with 20% ammonium sulphate. After overnight dialysis vs 20mM Tris Hcl pH7.5, 100mM NaCl to remove ammonium sulphate, the protein was further purified on Ni-NTA resin as described above. The VEGF domain protein was expressed in insoluble form, and preparation of

35

inclusion bodies was carried out using standard procedures (Ausubel et al 1997). Inclusion bodies were dissolved in 6M guanidine hydrochloride, 20mM Tris HCl pH8.0, 200mM NaCl, 1mM 2-mercaptoethanol, and purified on Ni-NTA resin (Qiagen GmbH, Düsseldorf, Germany) according to the manufacturer's instructions. The protein was refolded by dialysis against several changes of buffer containing decreasing concentrations of denaturant.

Analysis of protein glycosylation was carried out using EndoH (Roche Molecular Biochemicals, Brussels, BE) according to the manufacturer's instructions.

Cell Proliferation Assay

Human umbilical vein endothelial cells (HUVECs) (Clonetics, San Diego, CA.) were trypsinized with 0.05% trypsin/0.53mM EDTA (Gibco, Gaithersburg, MD.), resuspended in the EGM-2 (Clonetics, San Diego, CA.), counted, and distributed in a 96-well tissue culture plate at 5,000 cells/well. Following cell attachment and monolayer formation (16 hours), cells were stimulated with various concentrations of truncated VEGF-X (CUB domain or VEGF domain) or dilutions of culture supernatants of the full-length VEGF-X (COS 7 or HEK293) in DMEM (Gibco, Gaithersburg, MD.) containing 0.5% to 2% FBS (HyClone, Logan, UT) as indicated. For human fetal dermal fibroblasts (American Type Culture Collection, Rockville, MD.), the growth medium was replaced by DMEM containing 0.1% BSA (Sigma, St. Louise, MO.) with or without various concentrations of truncated VEGF-X proteins. For HCASMC (Clonetics, San Diego, CA.), the medium was replaced by DMEM containing 0.5% FBS. The cells were treated for a further 24 hr-72 hr. For the measurement of proliferation, the culture media were replaced with 100 µl of DMEM containing 5% FBS and 3

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5 μ Ci/ml of [3H]-thymidine (Amersham, Arlington Heights, IL.). Following pulse labeling, cells were fixed with methanol/acetic acid (3:1, vol/vol) for 1 hour at room temperature. The cells were washed
10 twice with 250 μ l/well of 80% methanol. The cells were solubilized in 0.05% trypsin (100 μ l/well) for 30 minutes then in 0.5% SDS (100 μ l/well) for another 30 minutes. Aliquots of cell lysates (180 μ l) were combined with 2 ml of scintillation cocktail (Fisher, Springfield, NJ) and the radioactivity of cell
15 lysates was measured using a liquid scintillation counter (Wallac 1409). In each case, samples were performed in quadruplicate.

15 Chemotaxis Assay

 The chemotactic response of HUVECs was assayed using a 48-well modified Boyden chamber (NeuroProbe, Cabin John, MD.) and collagen-coated (0.1mg/ml type I collagen, Collaborative Biomedical, Bedford, MA.)
20 polycarbonate membrane filters with a pore diameter of 8 μ m (NeuroProbe, Cabin John, MD.). Cell suspensions (15,000/well) were loaded to the upper part of the chemotaxis chamber and stimulated for 4 hours with rhVEGF₁₆₅ (0.1-10 ng/ml) (Calbiochem, San
25 Diego, CA.) or various concentrations of truncated VEGF-X (PDGF domain). Cells remaining on the top of the membrane were removed. Migration was assessed by counting the number of cells that migrated to the lower side of the filter membrane. The membrane was
30 fixed with 10% formaldehyde for 15 min, followed by staining with Gill's hemotoxylin III (Poly Scientific, Bay Shore, NY.). The assay was performed in triplicates and six independent high power fields per well were counted using a light microscope at 250
35 magnification. The results were expressed as the fold of unstimulated cells (EGM containing 0.1% BSA).

In Vitro Angiogenesis Assay

In vitro angiogenesis in fibrin gels was quantitated using spheroids of human umbilical vein endothelial cells (Korff et al., 1998). To generate endothelial cell spheroids of defined size and cell number, a specific number of cells (~ 800 cells per spheroid) was suspended in EGM-2 culture medium containing 20% methylcellulose (Sigma, St. Louis, MO.), seeded into nonadherent round-bottom 96-well plates. All suspended cells in one well contributed to the formation of a single endothelial cell spheroid within 24 hours. A fibrin gel stock solution was prepared freshly prior to use by mixing 3mg/ml fibrinogen (Calbiochem, San Diego, CA.) in Medium 199 (Gibco, Gaithersburg, MD.). Assays were performed in 24-well culture plates. The 1ml fibrinogen stock was mixed with 50 HUVEC spheroids and the corresponding test substance including rh-VEGF₁₆₅ or various concentration of VEGF-X. The spheroid-containing fibrinogen was rapidly transferred into 24-well plates. Fifteen microliters of thrombin (100 NIH U/ml stock, Sigma, St. Louis, MO.) was added to the gel for the fibrin gel formation. The gel formation usually occurred within 30 seconds. After gel formation, 1ml/well of Medium 199 supplemented with 20% FBS, 1mg/ml ϵ -aminocaproic acid (Calbiochem, San Diego, CA.) and antibiotics were added. The gel was incubated at 37°C (5%CO₂, 95% air, 100% humidity). After 3 days, *in vitro* angiogenesis was quantitated by measuring the length of the three longest capillary sprouts that had grown out of each spheroid (100X magnification), analyzing at least 10 spheroids per experimental group and experiment.

Matrigel Mouse Assay

The matrigel mouse assay is carried out as described by Passanti et al (1992).

Analysis of VEGF-X gene expression by RT-PCR analysis.

5 Oligonucleotide primers VEGF-E2 and VEGF-X14 (figure 16; figure 5) were used for the specific PCR amplification of a 350 bp fragment from VEGF-X. PCR amplifications were performed on human multiple
10 tissue cDNA (MTC™) panels (Clontech human MTC panels I and II and human Tumor MTC panel) normalised to the mRNA expression levels of six different housekeeping genes. In addition, cDNA was made from different tumor cell cultures (Caco-2 colorectal
15 adenocarcinoma; T-84 colorectal carcinoma; MCF-7 breast adenocarcinoma; T-47D breast ductal gland carcinoma; HT1080 bone fibrosarcoma; SaOS-2 osteosarcoma; SK-N-MC neuroblastoma; HepG2 hepatoblastoma; JURKAT T-cell leukemia and THP-1
20 myelomonocytic leukemia). For the preparation of tumor cell cDNA, cells were homogenised and total RNA prepared using the RNeasy Mini kit (Qiagen GmbH, Hilden, Germany) according to manufacturer's instructions. 1 µg of total RNA was reverse
25 transcribed using oligo(dT)15 as a primer and 50 U of Expand™ Reverse Transcriptase (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer's instructions. PCR reactions with VEGF-X-specific or glyceraldehyde-3-phosphate dehydrogenase
30 (G3PDH)-specific primers were then performed on 1 µl of this cDNA. For all cDNAs, PCR reactions with VEGF-X specific primers were performed in a total volume of 50 µl, containing 5 µl (± 1 ng) of cDNA, 1x Advantage KlenTaq PCR reaction buffer, 0.2 mM dNTP, 250 nM of primers VEGF-E2 and VEGF-X14 and 1 µl of
35 Advantage KlenTaq polymerase mix. Samples were heated

to 95°C for 30 s and cycling was done for 30 s at 95°C and 30 s at 68°C for 25, 30 or 35 cycles. Control reactions using specific primers that amplify a 1 kb fragment of the housekeeping gene G3PDH were also performed according to the manufacturer's instructions.

Northern blot analysis of VEGF-X.

Northern blots containing 2 µg of poly(A)-rich RNA derived from different human tissues (Clontech Laboratories; MTN™ blot, MTN™ blot II and Cancer Cell Line MTN™ blot) were hybridized according to the manufacturers instructions with a α -[³²P]-dCTP random-priming labelled (Multiprime labelling kit, Roche Diagnostics) 293 bp specific VEGF-X fragment (PinAI-StuI fragment including 92 bp of the 3' end coding region and 201 bp of the 3' untranslated region of VEGF-X). The blots were hybridized overnight at 68°C and final washes at high stringency were at 68°C in 0.1x SSC/0.1 % SDS. The membranes were autoradiographed for 1 to 3 days with intensifying screens.

Full length VEGF-X

The effect of full length VEGF-X on proliferation of HuVEC cells was determined by the ³H-Thymidine incorporation assay. HuVEC cells were serum starved for 24 hours prior to treatment with the full length VEGF-X at the concentration range from 100 pg/ml-10 µg/ml. There was no effect of VEGF-X at 100 pg/ml-10 ng/ml on endothelial cell proliferation. At the higher concentrations of FL-VEGF-X (100 ng/ml and 1 µg/ml) there was a marked inhibition of endothelial cell proliferation. This is probably due to the very high endotoxin level in the samples. The VEGF-X sample was purified in order to decrease the

endotoxin level and is currently tested in the cell proliferation assay.

The Summary from Testing the CUB Domain

5 The effect of CUB domain on inhibition of HuVEC proliferation either serum- (2%), rh-VEGF or bFGF-stimulated, was assessed by the ³H-Thymidine incorporation assay. Cells were serum starved followed by the treatment with the CUB domain and
10 various growth factors. Results showed that the CUB domain inhibited endothelial cell proliferation, either serum- (2%), rh-VEGF or bFGF-stimulated in a dose dependent manner with maximal inhibition at 10 µg/ml. There was approximately a 2-fold inhibition
15 of proliferation (at 10 µg/ml) of cells stimulated with VEGF and bFGF and nearly a 5-fold inhibition of cells stimulated with serum (2%). Results with the LDH assay showed that there was no cytotoxicity associated with the inhibition of cell proliferation
20 by the CUB domain.

Therefore, the N-terminus of the polypeptide from Figure 10 has been shown to possess a CUB domain. When database searches are carried out using the
25 full-length coding sequence the best matches (i.e. for a BLAST search, those with the lowest probability score) are found with the CUB domain rather than with the VEGF-like domain. The best match from searching release 37 of the SWISSPROT database (Feb 1999) is to
30 the CUB domain of a neuropilin from *Xenopus laevis*, and the matches to the CUB domains of human neuropilins 1 and 2 are also more significant than matches to the VEGFs.

35 This similarity is provocative, given the identification of neuropilin-1 and -2 as cellular receptors for the VEGF-A 165 (Stoker et al. 1998,

reviewed in Neufeld et al. 1999). It is plausible therefore that VEGF-X could exert dual regulatory effects: via interaction with the tyrosine kinase VEGF-receptors mediated by the VEGF-like domain, as well as via interaction with VEGF isoforms or with the neuropilin receptors, mediated by the CUB domain.

To the best of our understanding the latter would be entirely novel, and searches on the most recent release of the Incyte database do not reveal any other proteins containing both CUB and VEGF-like domains. This arrangement of domains suggests possible positive or negative models of regulation:

Positive- the VEGF-like domain is able to interact productively with the tyrosine kinase VEGF receptors giving activation, and the CUB domain is able to interact productively with the neuropilin receptor giving activation.

Negative- the VEGF-like domain does not interact productively with the tyrosine kinase VEGF receptors, either preventing receptor dimerisation or blocking the VEGF binding sites. Further, the CUB domain does not interact productively with the neuropilin receptors, either preventing receptor activation or blocking the VEGF binding sites, or indeed by binding to VEGF isoforms and preventing their interaction with receptors.

TABLE 1

	<u>ORIGINAL RESIDUE</u>	<u>EXEMPLARY SUBSTITUTIONS</u>
	ALA	SER, THR
5	ARG	LYS
	ASN	HIS, SER
	ASP	GLU, ASN
	CYS	SER
	GLN	ASN, HIS
10	GLU	ASP, GLU
	GLY	ALA, SER
	HIS	ASN, GLN
	ILE	LEU, VAL, THR
	LEU	ILE, VAL
15	LYS	ARG, GLN, GLU, THR
	MET	LEU, ILE, VAL
	PHE	LEU, TYR
	SER	THR, ALA, ASN
	THR	SER, ALA
20	TRP	ARG, SER
	TYR	PHE
	VAL	ILE, LEU ALA
	PRO	ALA

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5

10

SEQUENCE LISTING

5	Sequence ID No 1	corresponds to the amino acid sequence from position 23 to 345 of the amino acid sequence illustrated in Figure 10.
10	Sequence ID No 2	is the amino acid sequence illustrated in Figure 10.
15	Sequence ID No 3	corresponds to the sequence from position 257 to 1291 of the nucleotide sequence illustrated in Figure 9.
20	Sequence ID No 4	corresponds to the polynucleotide sequence of VEGFX1 illustrated in Figure 3.
25	Sequence ID No 5	corresponds to the polynucleotide sequence of VEGFX2 illustrated in Figure 3.
30	Sequence ID No 6	corresponds to the polynucleotide sequence of VEGFX3 illustrated in Figure 3.
35	Sequence ID No 7	corresponds to the polynucleotide sequence of VEGFX4 illustrated in Figure 3.
	Sequence ID No 8	corresponds to the polynucleotide sequence of VEGFX5 illustrated in Figure 3.
	Sequence ID No 9	corresponds to the polynucleotide sequence of VEGFX6 illustrated in

Figure 3.

5	Sequence ID No 10	corresponds to the polynucleotide sequence of VEGFX7 illustrated in Figure 3.
10	Sequence ID No 11	corresponds to the polynucleotide sequence of VEGFX8 illustrated in Figure 3.
	Sequence ID No 12	corresponds to the polynucleotide sequence of VEGFX9 illustrated in Figure 3.
15	Sequence ID No 13	corresponds to the polynucleotide sequence of VEGFX10 illustrated in Figure 3.
20	Sequence ID No 14	corresponds to the polynucleotide sequence of VEGFX11 illustrated in Figure 4.
25	Sequence ID No 15	corresponds to the polynucleotide sequence of VEGFX12 illustrated in Figure 4.
30	Sequence ID No 16	corresponds to the polynucleotide sequence of VEGFX13 illustrated in Figure 4.
	Sequence ID No 17	corresponds to the polynucleotide sequence of VEGFX14 illustrated in Figure 4.
35	Sequence ID No 18	corresponds to the polynucleotide sequence 5'-1 in Figure 8.

- Sequence ID No 19 corresponds to the polynucleotide sequence 5'-2 in Figure 8.
- 5 Sequence ID No 20 corresponds to the polynucleotide sequence of VEGFX6 illustrated in Figure 13.
- 10 Sequence ID No 21 corresponds to the polynucleotide sequence of VEGFX7 illustrated in Figure 13.
- 15 Sequence ID No 22 corresponds to the polynucleotide sequence of VEGFX8 illustrated in Figure 13.
- 20 Sequence ID No 23 corresponds to the polynucleotide sequence of VEGFX9 illustrated in Figure 13.
- 25 Sequence ID No 24 corresponds to the polynucleotide sequence of VEGBAC1 illustrated in Figure 13.
- 30 Sequence ID No 25 corresponds to the polynucleotide sequence of VEGBAC2 illustrated in Figure 13.
- 35 Sequence ID No 26 corresponds to a polypeptide having the amino acid sequence from amino acid position 40 to 150 of the sequence of Figure 10.
- Sequence ID No 27 corresponds to a polypeptide having the amino acid sequence illustrated in Figure 26.
- Sequence ID No 28 corresponds to the sequence from

position 5 to 508 of the
nucleotide sequence illustrated
in Figure 26.

- 5 Sequence ID No 29 corresponds to the nucleotide
sequence from position 5 to 508
of the nucleotide sequence
illustrated in Figure 26.
- 10 Sequence ID No 30 corresponds to the sequence from
position 214 to 345 of the
nucleotide sequence illustrated
in Figure 10.

CLAIMS

1. A nucleic acid molecule encoding a VEGF-X protein or a functional equivalent, derivative or bioprecursor thereof, said protein comprising any of the sequences from position 23 to 345 of the amino acid sequence illustrated in Figure 10, or the complete sequence as illustrated in Figure 10.
2. A nucleic acid molecule according to claim 1 wherein said nucleic acid is a DNA molecule.
3. A nucleic acid molecule according to claim 1 or 2 wherein said nucleic acid is a cDNA molecule.
4. A nucleic acid molecule according to claim 3 comprising the nucleotide sequence from position 257 to 1291 of the nucleotide sequence illustrated in Figure 9, or sequences that hybridise thereto under high stringency conditions or the complement thereto.
5. An antisense molecule capable of hybridising to a molecule according to any of claims 1 to 4 under high stringency conditions.
6. A nucleic acid molecule according to any of claims 1 to 4 which is of mammalian origin.
7. A nucleic acid molecule according to claim 6 which is of human origin.
8. An isolated VEGF-X protein, or a functional equivalent, derivative or bioprecursor thereof, having an amino acid sequence from position 23 to 345 of the amino acid sequence illustrated in Figure 10 or the complete amino acid sequence of Figure 10.

9. A VEGF-X protein, or a functional equivalent, derivative or bioprecursor thereof, encoded by a nucleic acid molecule as defined in any of claims 1 to 4.
- 5
10. A protein according to claim 9, which comprises the amino acid sequence illustrated in Figure 10.
- 10
11. An expression vector comprising a nucleic acid molecule according to any of claims 1 to 4.
- 15
12. An expression vector according to claim 11 further comprising a nucleotide sequence encoding a reporter molecule.
- 15
13. An expression vector comprising an antisense molecule according to claim 5.
- 20
14. A nucleic acid molecule according to any of claims 1 to 4 or an antisense molecule according to claim 5 for use as a medicament.
- 25
15. A host cell transformed or transfected with an expression vector according to claim 11 or 12.
- 25
16. A host cell transformed or transfected with an expression vector according to claim 13.
- 30
17. A transgenic cell, tissue or organism comprising a transgene capable of expressing a VEGF-X protein according to claim 8 or 9.
- 35
18. A transgenic cell, tissue or organism according to claim 17, wherein said transgene is included in an expression vector.
19. A VEGF-X protein or a functional equivalent,

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derivative or bioprecursor thereof, expressed by a cell according to claim 15.

5 20. A VEGF-X protein, or a functional equivalent, derivative or bioprecursor thereof, expressed by a transgenic cell, tissue or organism according to claim 17.

10 21. A process for producing a VEGF-X protein according to any of claims 8 to 10, said process comprising transforming a host cell or organism with an expression vector according to claim 11, and recovering the expressed protein from said host cell or organism.

15 22. An antibody capable of binding to a protein according to any of claims 8 to 10, or an epitope thereof.

20 23. An antibody according to claim 22 for use as a medicament.

25 24. A pharmaceutical composition comprising an antibody according to claim 22 together with a pharmaceutically acceptable carrier diluent or excipient thereof.

30 25. A method of identifying VEGF-X protein in a sample which method comprises contacting said sample with an antibody according to claim 22 and monitoring for binding of any protein to said antibody.

35 26. A kit for identifying the presence of VEGF-X protein in a sample which comprises an antibody according to claim 22 and means for contacting said antibody with said sample.

27. A method of identifying compounds which modulate angiogenesis which method comprises providing a host cell or organism according to claim 15 or a transgenic cell, tissue or organism according to claim 17, contacting a test compound with said cell, tissue or organism and monitoring for an effect of said compound on said VEGF compared to a host cell or organism according to claim 15 or a transgenic cell tissue or organism according to claim 17 which has not been contacted with said compound.
28. A compound identifiable according to the method of claim 27.
29. A compound according to claim 28 for use as a medicament.
30. A nucleic acid sequence comprising the nucleotide sequences illustrated in any of Figures 3, 5, 8 or 13.
31. A method for producing a polypeptide, said method comprising the steps of:
- a) culturing the host cell of claim 15 under conditions suitable for expression of the polypeptide; and
 - b) recovering the polypeptide from the host cell culture.
32. A method of inhibiting angiogenic activity and inappropriate vascularisation including formation and proliferation of new blood vessels, growth and development of tissues, tissue regeneration and organ and tissue repair in a subject said method comprising administering to said subject an amount of an antisense molecule according to claim 5 in sufficient

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concentration to reduce or prevent said angiogenic activity.

5 33. A method of inhibiting angiogenic activity or inappropriate vascularisation including any of formation and proliferation of new blood vessels, growth and development of tissues, tissue regeneration and organ and tissue repair in a subject
10 said method comprising administering to said subject an amount of an antibody according to claim 22 in sufficient concentration to reduce or prevent said angiogenic activity or inappropriate vascularisation.

15 34. A method of inhibiting angiogenic activity or inappropriate vascularisation including any of formation and proliferation of new blood vessels, growth and development of tissues, tissue regeneration and organ and tissue repair in a
20 subject, said method comprising implanting in said subject cells that express an antibody according to claim 22.

25 35. A method of treating or preventing any of cancer, rheumatoid arthritis, psoriasis and diabetic retinopathy, said method comprising administering to said subject an amount of an antisense molecule according to claim 5 in sufficient concentration to treat or prevent said disorders.

30 36. A method of treating or preventing any of cancer, rheumatoid arthritis, psoriasis and diabetic retinopathy, said method comprising administering to said subject an amount of an antibody according to claim 22 in sufficient concentration to reduce or
35 prevent said disorders.

37. A method of promoting angiogenic activity or

- vascularisation to promote wound healing, skin graft growth, tissue repair, proliferation of new blood vessels, tissue regeneration and organ repair which method comprises applying or delivering to a site of interest a therapeutically effective amount of any of a group selected from a protein according to claim 8 and a nucleic acid molecule encoding a VEGF-X protein or a functional equivalent, derivative or bioprecursor thereof comprising an amino acid sequence illustrated in Figure 10, an expression vector comprising said nucleic acid molecule and a pharmaceutical composition comprising any of said nucleic acid molecule and said protein.
38. A method of treating wounds selected from the group consisting of dermal ulcers, pressure sores, venous sores, diabetic ulcers and burns by applying to said wound a therapeutically effective amount of any of a VEGF-X protein according to claim 8, a pharmaceutical composition comprising said protein and a pharmaceutically acceptable carrier, diluent or excipient therefor.
39. A nucleic acid molecule encoding a polypeptide having a CUB domain said polypeptide comprising the amino acid sequence from position 40 to 150 of the sequence of Figure 10.
40. A nucleic acid molecule encoding a polypeptide having a CUB domain, said polypeptide comprising the amino acid sequence of Figure 26.
41. A nucleic acid molecule according to claim 39 or 40, comprising the nucleotide sequence from position 5 to 508 of the sequence illustrated in Figure 26.
42. A nucleic acid molecule according to any of

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claims 39 to 41 comprising the nucleotide sequence illustrated in Figure 26.

5 43. A nucleic acid molecule encoding a VEGF like domain comprising the sequence from position 214-345 of the sequence of Figure 10 or the sequence from position 15 to 461 illustrated in Figure 24.

10 44. An expression vector comprising a nucleic acid molecule according to any of claims 39 to 42.

45. An expression vector comprising a nucleic acid molecule according to claim 43.

15 46. A host cell transformed or transfected with an expression vector according to claim 44.

20 47. A host cell transformed or transfected with an expression vector according to claim 45.

48. A protein expressed by the cell according to claim 46.

25 49. A protein expressed by the cell according to claim 47.

30 50. A method of identifying compounds that inhibit or enhance angiogenic activity, said method comprising contacting a cell expressing a VEGF receptor and/or a neuropilin 1 or 2 type receptor with said compound in the presence of a VEGF-X protein according to claim 8 and monitoring for the effect of said compound or said cell when compared to a cell which has not been contacted with said
35 compound.

51. A compound identifiable according to the method

of claim 50 as an inhibitor or enhancer of angiogenic activity.

5 52. A method of inhibiting angiogenic activity or inappropriate vascularisation, said method comprising contacting a cell expressing a VEGF receptor and a neuropilin type receptor with a protein selected from any of a protein according to any of claims 8 to 10 and a protein according to claim 48 or a protein
10 according to claim 49.

15 53. Use of a nucleotide sequence illustrated in any of Figures 14 and 15 in identifying a VEGF-X protein according to claim 8.

54. A nucleic acid molecule encoding a polypeptide comprising a CUB domain having the sequence from position 40 to 150 of the sequence of Figure 10 or from position 5 to 508 of the sequence of Figure 26
20 and a sequence encoding a VEGF domain.

25 55. A nucleic acid molecule according to claim 54 wherein said sequence encoding said VEGF domain is selected from the sequences encoding any of VEGF A to D or isoforms or variants thereof.

30 56. A nucleic acid molecule encoding a polypeptide comprising the amino acid sequence from position 40 to 150 of the sequence illustrated in Figure 10 for use as a medicament.

35 57. Use of a nucleic acid molecule encoding a polypeptide having the amino acid sequence from position 40 to 150 of the sequence illustrated in Figure 10 in the manufacture of a medicament for treatment of disease conditions associated with inappropriate angiogenesis such as tumour or cancer

growth, retinopathy, osteoarthritis or psoriasis.

58. A polypeptide comprising the amino acid sequence
from position 40 to 150 of the sequence illustrated
5 in figure 10 for use as a medicament.

59. A polypeptide comprising the amino acid sequence
from position 40 to 150 of the sequence illustrated
in Figure 10 in the manufacture of a medicament for
10 the treatment of disease conditions associated with
inappropriate angiogenesis such as tumour growth,
retinopathy, osteoarthritis or psoriasis.

60. Use of a CUB domain comprising the amino acid
15 sequence from position 40 to 150 of the sequence of
Figure 10, or the amino acid sequence of Figure 26,
to identify compounds which inhibit angiogenic
activity in a method according to claim 50.

61. A method of inhibiting angiogenic activity and
inappropriate vascularisation including formation and
proliferation of new blood vessels, growth and
development of tissues, tissue regeneration and organ
and tissue repair in a subject said method comprising
25 administering to said subject an amount of a
polypeptide having an amino acid sequence from
position 40 to 150 of the sequence illustrated in
Figure 10 or a nucleic acid molecule according to any
of claims 39 to 42 in sufficient concentration to
30 reduce or prevent said angiogenic activity.

62. A method of treating or preventing any of
cancer, rheumatoid arthritis, psoriasis and diabetic
retinopathy, said method comprising administering to
35 said subject an amount of a polypeptide having an
amino acid sequence from position 40 to 150 of the
sequence illustrated in Figure 10 or a nucleic acid

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molecule according to any of claims 39 to 42 in sufficient concentration to treat or prevent said disorders.

- 5 63. An antisense molecule capable of hybridising to a molecule according to any of claims 39 to 42 under high stringency conditions.
- 10 64. An antisense molecule capable of hybridising to a molecule according to claim 43 under high stringency conditions.
- 15 65. A transgenic cell, tissue or organism comprising a transgene capable of expressing a protein according to claim 48.
- 20 66. A transgenic cell, tissue or organism comprising a transgene capable of expressing a protein according to claim 49.
- 25 67. A transgenic, cell tissue or organism according to claim 65 or 66, wherein said transgene is included in an expression vector according to claim 41 or 42.
- 30 68. An antibody capable of binding to a protein according to claim 48 or an epitope thereof.
- 35 69. An antibody capable of binding to a protein according to claim 49 or an epitope thereof.
70. A pharmaceutical composition comprising an antibody according to claim 68 or 69 together with a pharmaceutically acceptable carrier diluent or excipient therefor.
71. A pharmaceutical composition comprising a

compound according to claim 48 together with a pharmaceutically acceptable carrier, diluent or excipient therefor.

- 5 72. A nucleic acid molecule encoding a variant of a VEGF-X protein having any of the sequences of nucleotides illustrated in Figure 12.

FIG. 1

1 AAAATGTATG GATACAACTT ACGTTTGATG AAAGATTTGG GCTTGAAGAC CCAGAAGATG
 TTTTACATAC CTATGTTGAA TGCAAACCTAC TTTCTAAACC CGAACTTCTG GGTCTTCTAC
 61 ACATATGCAA GTATGATTTT GTAGAAGTTG AGGAACCCAG TGATGGAACCT ATATTAGGGC
 TGTATACGTT CATACTAAAA CATCTTCAAC TCCTTGGGTC ACTACCTTGA TATAATCCCG
 121 GCTGGTGTGG TTCTGGTACT GTACCAGGAA AACAGATTTC TAAAGGAAAT CAAATTAGGA
 CGACCACACC AAGACCATGA CATGGTCTT TTGTCTAAAG ATTCCTTTA GTTTAATCCT
 +1 MetAsn IlePheLeu LeuAsnLeuLeu ThrGluGlu ValArgLeu
]-----
 181 TAAGATTTGT ATCTGATGAA TATTTTCCTT CTGAACCTTC TAACAGAGGA GGTAAGATTA
 ATTCTAAACA TAGACTACTT ATAAAAGGAA GACTTGGAAG ATTGTCTCCT CCATTCTAAT
 +1 TyrSerCysThr ProArgAsn PheSerVal SerIleArgGlu GluLeuLys ArgThrAsp

 241 TACAGCTGCA CACCTCGTAA CTTCTCAGTG TCCATAAGGG AAGAACTAAA GAGAACCGAT
 ATGTCGACGT GTGGAGCATT GAAGAGTCAC AGGTATTCCC TTCTTGATTT CTCTGGGCTA
 +1 ThrIlePheTrp ProGlyCys LeuLeuVal LysArgCysGly GlyAsnCys AlaCysCys

 301 ACCATTTTCT GGCCAGGTTG TCTCCTGGTT AAACGCTGTG GTGGGAACCTG TGCCTGTTGT
 TGGTAAAAGA CCGGTCCAAC AGAGGACCAA TTGCGACAC CACCCTTGAC ACGGACAACA
 +1 LeuHisAsnCys AsnGluCys GlnCysVal ProSerLysVal ThrLysLys TyrHisGlu

 361 CTCCACAATT GCAATGAATG TCAATGTGTC CCAAGCAAAG TTAATAAAAA ATACCACGAG
 GAGGTGTTAA CGTTACTTAC AGTTACACAG GGTTCTGTTT AATGATTTT TATGGTGCTC
 +1 ValLeuGlnLeu ArgProLys ThrGlyVal ArgGlyLeuHis LysSerLeu ThrAspVal

 421 GTCCTTCAGT TGAGACCAAA GACCGGTGTC AGGGGATTGC ACAAATCACT CACCGACGTG
 CAGGAAGTCA ACTCTGGTTT CTGGCCACAG TCCCCTAACG TGTTTAGTGA GTGGCTGCAC
 +1 AlaLeuGluHis HisGluGlu CysAspCys ValCysArgGly SerThrGly Gly
 ----->
 481 GCCCTGGAGC ACCATGAGGA GTGTGACTGT GTGTGCAGAG GGAGCACAGG AGGATAGCCG
 CGGGACCTCG TGGTACTCCT CACACTGACA CACACGTCTC CCTCGTGTCC TCCTATCGGC
 541 CATCACCACC AGCAGCTCTT GCCCAGAGCT GTGCAGTGCA GTGGCTGATT CTATTAGAGA
 GTAGTGGTGG TCGTCGAGAA CGGGTCTCGA CACGTCACGT CACCGACTAA GATAATCTCT
 601 ACGTATGCGT TATCTCCATC CTTAATCTCA GTTGTGCTGCT TCAAGGACCT TTCATCTTCA
 TGCATACGCA ATAGAGGTAG GAATTAGAGT CAACAAACGA AGTTCCTGGA AAGTAGAAGT
 661 GGATTTACAG TGCATTCTGA AAGAGGAGAC ATCAAACAGA ATTAGGAGTT GTGCAACAGC
 CCTAAATGTC ACGTAAGACT TTCTCTCTG TAGTTTGTCT TAATCCTCAA CACGTTGTCC
 721 TCTTTTGA GAAGGCTTAA AGGACAGGAG AAAAGGTCTT CAATCGTGGA AAGAAAATTA
 AGAAAACCTCT CCTCCGATT TCCTGTCTC TTTTCCAGAA GTTAGCACCT TTCTTTAAT
 781 AATGTTGTAT TAAATAGATC ACCAGCTAGT TTCAGAGTTA CCATGTACGT ATTCCACTAG
 TTACAACATA ATTTATCTAG TGGTCGATCA AAGTCTCAAT GGTACATGCA TAAGGTGATC

FIG. 1 (CONTINUED).

841 CTGGGTTCTG TATTCAGTT CTTTCGATAC GGCTTAGGGT AATGTCAGTA CAGGAAAAAA
GACCCAAGAC ATAAAGTCAA GAAAGCTATG CCGAATCCCA TTACAGTCAT GTCCTTTTTT

901 ACTGTGCAAG TGAGCACCTG ATTCCGTTGC CTTGCTTAAC TCTAAAGCTC CATGTCCTGG
TGACACGTTT ACTCGTGGAC TAAGGCAACG GAACGAATTG AGATTTCGAG GTACAGGACC

961 GCCTAAAATC GTATAAAATC TGGATTTTTT TTTTTTTTTT TGCTCATATT CACATATGTA
CGGATTTTAG CATATTTTAG ACCTAAAAAA AAAAAAAAAA ACGAGTATAA GTGTATACAT

1021 AACCAGAACA TTCTATGTAC TACAAACCTG GTTTTTAAAA AGGAACTATG TTGCTATGAA
TTGGTCTTGT AAGATACATG ATGTTTGGAC CAAAAATTTT TCCTTGATAC AACGATACTT

1081 TTAAACTTGT GTCGTGCTGA TAGGACAGAC TGGATTTTTC ATATTTCTTA TTAAAATTTT
AATTTGAACA CAGCAGACT ATCCTGTCTG ACCTAAAAAG TATAAAGAAT AATTTTAAAG

1141 TGCCATTTAG AAGAAGAGAA CTACATTCAT GGTTTGGAAG AGATAAACCT GAAAAGAAGA
ACGGTAAATC TTCTTCTCTT GATGTAAGTA CCAAACCTTC TCTATTGGA CTTTCTTCTT

1201 GTGGCCTTAT CTTCACTTTA TCGATAAGTC AGTTTATTTG TTTCATTGTG TACATTTTTA
CACCGGAATA GAAGTGAAAT AGCTATTCTG TCAAATAAAC AAAGTAACAC ATGTAAAAAT

1261 TATTCCTCTT TTGACATTAT AACTGTTGGC TTTTCTAATC TTGTTAAATA TATCTATTTT
ATAAGAGGAA AACTGTAATA TTGACAACCG AAAAGATTAG AACAATTTAT ATAGATAAAA

1321 TACCAAAGGT ATTTAATATT CTTTTTATG ACAACTTAGA TCACTATTTT TTAGCTTGGT
ATGGTTTCCA TAAATTATAA GAAAAAATAC TGTGAATCT AGTTGATAAA AATCGAACCA

1381 AAATTTTCTT AAACACAATT GTTATAGCCA GAGGAACAAA GATGATATAA AATATTGTTG
TTTAAAAAGA TTTGTGTAA CAATATCGGT CTCCTTGTTT CTACTATATT TTATAACAAC

1441 CTCTGACAAA AATACATGTA TTTCATTCTC GTATGGTGCT AGAGTTAGAT TAATCTGCAT
GAGACTGTTT TTATGTACAT AAAGTAAGAG CATAACACGA TCTCAATCTA ATTAGACGTA

1501 TTTAAAAAAC TGAATTGGAA TAGAATTGGT AAGTTGCAAA GACTTTTTGA AAATAATTAA
AAATTTTTTG ACTTAACCTT ATCTTAACCA TTCAACGTTT CTGAAAAACT TTTATTAATT

1561 ATTATCATAT CTTCATTCC TGTATTGGA GATGAAAATA AAAAGCAACT TATGAAAGTA
TAATAGTATA GAAGGTAAGG ACAATAACCT CTACTTTTAT TTTTCGTTGA ATACTTTCAT

1621 GACATTCAGA TCCAGCCATT ACTAACCTAT TCCTTTTTTG GGGAAATCTG AGCCTAGCTC
CTGTAAGTCT AGGTCGGTAA TGATTGGATA AGGAAAAAAC CCCTTTAGAC TCGGATCGAG

1681 AGAAAAACAT AAAGCACCTT GAAAAAGACT TGGCAGCTTC CTGATAAAGC GTGCTGTGCT
TCTTTTTGTA TTTCGTGGAA CTTTTTCTGA ACCGTCGAAG GACTATTTTC CACGACACGA

1741 GTGCAGTAGG AACACATCCT ATTTATTGTG ATGTTGTGGT TTTATTATCT TAACTCTGT
CACGTCATCC TTGTGTAGGA TAAATAACAC TACAACACCA AAATAATAGA ATTTGAGACA

1801 TCCATACACT TGTATAAATA CATGGATATT TTTATGTACA GAAGTATGTC TCTTAACCAG
AGGTATGTGA ACATATTTAT GTACCTATAA AAATACATGT CTTCATACAG AGAATTGGTC

1861 TTCACTTATT GTACCTGG
AAGTGAATAA CATGGACC

FIG. 2. Predicted VEGF-like protein encoded by Incyte contig of 8/12/98

1 MNIFLLNLLT EEVRLYSCTP RNFSVSIREE LKRTDTIFWP GCLLVKRCGG
51 NCACCLHNCN ECQCVPSKVT KKYHEVLQLR PXTGVRGLHX SLTDVALEHH
101 EECDCVCRGS TGG

FIG. 3. PCR primers for cloning VEGF-X

vegFX1	AAAATGTATGGATACAACTTAC
vegFX2	GTTTGATGAAAGATTTGGGCTTG
vegFX3	TTTCTAAAGGAAATCAAATTAG
vegFX4	GATAAGATTTGTATCTGATG
vegFX5	GATGTCTCCTCTTTTCAG
vegFX6	GCACAACTCCTAATTCTG
vegFX7	AGCACCTGATTCCGTTGC
vegFX8	TAGTACATAGAATGTTCTGG
vegFX9	AAGAGACATACTTCTGTAC
vegFX10	CCAGGTACAATAAGTGAAGTGA



FIG. 5. VEGF-X 5' RACE primers

vegfX11	CCTTTAGAAATCTGTTTTCTGGTACAG
vegfX12	GGAAAATATTCATCAGATACAAATCTTATCC
vegfX13	GGTCCAGTGGCAAAGCTGAAGG
vegfX14	CTGGTTCAAGATATCGAATAAGGTCTTCC

FIG. 6. DNA sequence assembled from in-house clones and 5'RACE

```

1  TGCCAGAGCA GGTGGGCGCT TCCACCCAG TGCAGCCTTC CCCTGGCGGT GGTGAAAGAG
   ACGGTCTCGT CCACCCGCGA AGGTGGGGTC ACGTCGGAAG GGGACCGCCA CCACTTTCTC

61  ACTCGGGAGT CGCTGCTTCC AAAGTGCCCG CCGTGAGTGA GCTCTACCC CAGTCAGCCA
   TGAGCCCTCA GCGACGAAGG TTTCACGGGC GGCCTCACT CGAGAGTGGG GTCAGTCGGT

+2  MetSerLeu PheGlyLeuLeu LeuLeuThr SerAlaLeu AlaGlyGlnArg GlnGlyTh
   ]-----
121  AATGAGCCTC TTCGGGCTTC TCCTGCTGAC ATCTGCCCTG GCCGGCCAGA GACAGGGGAC
   TTACTCGGAG AAGCCCGAAG AGGACGACTG TAGACGGGAC CGGCCGGTCT CTGTCCCTG

+2  rGlnAlaGlu SerAsnLeuSer SerLysPhe GlnPheSer SerAsnLysGlu GlnAsnGl
   -----
181  TCAGGCGGAA TCCAACCTGA GTAGTAAATT CCAGTTTTCC AGCAACAAGG AACAGAACGG
   AGTCCGCCTT AGGTGGACT CATCATTTAA GGTCAAAGG TCGTTGTTCC TTGTCTGCC

+2  yValGlnAsp ProGlnHisGlu ArgIleIle ThrValSer ThrAsnGlySer IleHisSe
   -----
241  AGTACAAGAT CCTCAGCATG AGAGAATTAT TACTGTGTCT ACTAATGGAA GTATTCACAG
   TCATGTTCTA GGAGTCGTAC TCTCTTAATA ATGACACAGA TGATTACCTT CATAAGTCTC

+2  rProArgPhe ProHisThrTyr ProArgAsn ThrValLeu ValTrpArgLeu ValAlaVa
   -----
301  CCCAAGGTTT CCTCATACTT ATCCAAGAAA TACGGTCTTG GTATGGAGAT TAGTAGCAGT
   GGGTTCCAAA GGAGTATGAA TAGGTTCTTT ATGCCAGAAC CATACTCTA ATCATCGTCA

+2  lGluGluAsn ValTrpIleGln LeuThrPhe AspGluArg PheGlyLeuGlu AspProGl
   -----
361  AGAGGAAAAT GTATGGATAC AACTTACGTT TGATGAAAGA TTTGGGCTTG AAGACCCAGA
   TCTCCTTTTA CATACTATG TTGAATGCAA ACTACTTTCT AAACCCGAAC TTCTGGGTCT

+2  uAspAspIle CysLysTyrAsp PheValGlu ValGluGlu ProSerAspGly ThrIleLe
   -----
421  AGATGACATA TGCAAGTATG ATTTTGTAGA AGTTGAGGAA CCCAGTGATG GAACTATATT
   TCTACTGTAT ACGTTCATAC TAAAACATCT TCAACTCCTT GGGTCACTAC CTTGATATAA

+2  uGlyArgTrp CysGlySerGly ThrValPro GlyLysGln IleSerLysGly AsnGlnIl
   -----
481  AGGGCGCTGG TGTGGTTCTG GTACTGTACC AGGAAAACAG ATTTCTAAAG GAAATCAAAT
   TCCCGCGACC ACACCAAGAC CATGACATGG TCCTTTTGTC TAAAGATTTC CTTTAGTTTA

+2  eArgIleArg PheValSerAsp GluTyrPhe ProSerGlu ProGlyPheCys IleHisTy
   -----
541  TAGGATAAGA TTTGTATCTG ATGAATATTT TCCTTCTGAA CCAGGGTCTT GCATCCACTA
   ATCCTATTCT AAACATAGAC TACTTATAAA AGGAAGACTT GGTCCCAAGA CGTAGGTGAT

+2  rAsnIleVal MetProGlnPhe ThrGluAla ValSerPro SerValLeuPro ProSerAl
   -----
601  CAACATTGTC ATGCCACAAT TCACAGAAGC TGTGAGTCCT TCAGTGCTAC CCCCTTCAGC
   GTTGTAACAG TACGGTGTTA AGTGTCTTCG AACTCAGGA AGTCACGATG GGGGAAGTCG

+2  aLeuProLeu AspLeuLeuAsn AsnAlaIle ThrAlaPhe SerThrLeuGlu AspLeuIl
   -----
661  TTTGCCACTG GACCTGCTTA ATAATGCTAT AACTGCCTTT AGTACCTTGG AAGACCTTAT

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FIG. 6 (CONTINUED 1).

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+2 eArgTyrLeu GluProGluArg TrpGlnLeu AspLeuGlu AspLeuTyrArg ProThrTr
-----
721 TCGATATCTT GAACCAGAGA GATGGCAGTT GGAAGTAGAA GATCTATATA GGCCAACTTG
    AGCTATAGAA CTTGGTCTCT CTACCGTCAA CCTGAATCTT CTAGATATAT CCGGTTGAAC

+2 pGlnLeuLeu GlyLysAlaPhe ValPheGly ArgLysSer ArgValValAsp LeuAsnLe
-----
781 GCAACTTCTT GGCAAGGCTT TTGTTTTTGG AAGAAAATCC AGAGTGGTGG ATCTGAACCT
    CGTTGAAGAA CCGTTCCGAA AACAAAAACC TTCTTTTAGG TCTCACCACC TAGACTTGGA

+2 uLeuThrGlu GluValArgLeu TyrSerCys ThrProArg AsnPheSerVal SerIleAr
-----
841 TCTAACAGAG GAGGTAAGAT TATACAGCTG CACACCTCGT AACTTCTCAG TGTCCATAAG
    AGATTGTCTC CTCCATTCTA ATATGTCGAC GTGTGGAGCA TTGAAGAGTC ACAGGTATTCT

+2 gGluGluLeu LysArgThrAsp ThrIlePhe TrpProGly CysLeuLeuVal LysArgCy
-----
901 GGAAGAACTA AAGAGAACCG ATACCATTTT CTGGCCAGGT TGTCTCCTGG TTAAACGCTG
    CCTTCTTGAT TTCTCTTGGC TATGGTAAAA GACCGGTCCA ACAGAGGACC AATTTGCGAC

+2 sGlyGlyAsn CysAlaCysCys LeuHisAsn CysAsnGlu CysGlnCysVal ProSerLy
-----
961 TGGTGGGAAC TGTGCCTGTT GTCTCCACAA TTGCAATGAA TGTCAATGTG TCCCAAGCAA
    ACCACCCTTG ACACGGACAA CAGAGGTGTT AACGTTACTT ACAGTTACAC AGGGTTCTGTT

+2 sValThrLys LysTyrHisGlu ValLeuGln LeuArgPro LysThrGlyVal ArgGlyLe
-----
1021 AGTTACTAAA AAATACCACG AGGTCCTTCA GTTGAGACCA AAGACCGGTG TCAGGGGATT
    TCAATGATTT TTTATGGTGC TCCAGGAAGT CAACTCTGGT TTCTGGCCAC AGTCCCCTAA

+2 uHisLysSer LeuThrAspVal AlaLeuGlu HisHisGlu GluCysAspCys ValCysAr
-----
1081 GCACAAATCA CTCACCGACG TGGCCCTGGA GCACCATGAG GAGTGTGACT GTGTGTGCAG
    CGTGTTTAGT GAGTGGCTGC ACCGGGACCT CGTGGTACTC CTCACACTGA CACACACGTC

+2 gGlySerThr GlyGly
----->
1141 AGGGAGCACA GGAGGATAGC CGCATCACCA CCAGCAGCTC TTGCCAGAG CTGTGCAGTG
    TCCCTCGTGT CCTCCTATCG GCGTAGTGGT GGTCTGTCGAG AACGGGTCTC GACACGTAC

1201 CAGTGGCTGA TTCTATTAGA GAACGTATGC GTTATCTCCA TCCTTAATCT CAGTTGTTTTG
    GTCACCGACT AAGATAATCT CTTGCATACG CAATAGAGGT AGGAATTAGA GTCAACAAAC

1261 CTTCAAGGAC CTTTCATCTT CAGGATTTAC AGTGCATTCT GAAAGAGGAG ACATCAAACA
    GAAGTTCCTG GAAAGTAGAA GTCCTAAATG TCACGTAAGA CTTTCTCCTC TGTAGTTTGT

1321 GAATTAGGAG TTGTGCAACA GCTCTTTTGA GAGGAGGCCT AAAGGACAGG AGAAAAGGTC
    CTTAATCCTC AACACGTTGT CGAGAAAACCT CTCCTCCGGA TTTCTGTGCC TCTTTTCCAG

1381 TTCAATCGTG GAAAGAAAAT TAAATGTTGT ATTAAATAGA TCACCAGCTA GTTTCAGAGT
    AAGTTAGCAC CTTTCTTTTA ATTTACAACA TAATTTATCT AGTGGTGCAT CAAAGTCTCA

1441 TACCATGTAC GTATTCCACT AGCTGGGTTT TGTATTTTCTG TTTCTTCGAT ACGGCTTAGG
    ATGGTACATG CATAAGGTGA TCGACCCAAG ACATAAAGTC AAGAAAGCTA TGCCGAATCC

1501 GTAATGTCAG TACAGGAAAA AAAGTGTGCA AGTGAGCACC TGATTCCGTT GCCTTGCTTA
    -----

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FIG. 6 (CONTINUED 2).

1561 ACTCTAAAGC TCCATGTCCT GGGCCTAAAA TCGTATAAAA TCTGGATTTT TTTTTTTTTT
 TGAGATTTTCG AGGTACAGGA CCCGGATTTT AGCATATTTT AGACCTAAAA AAAAAAAAAA
 1621 TTTGCTCATA TTCACATATG TAAACCAGAA CATTCTATGT ACTACAAACC TGGTTTTTAA
 AAACGAGTAT AAGTGTATAC ATTTGGTCTT GTAAGATACA TGATGTTTGG ACCAAAAATT
 1681 AAAGGAACTA TGTTGCTATG AATTAACTT GTGTCGTGCT GATAGGACAG ACTGGATTTT
 TTTCTTGAT ACAACGATAC TTAATTTGAA CACAGCACGA CTATCCTGTC TGACCTAAAA
 1741 TCATATTTCT TATTAAAATT TCTGCCATTT AGAAGAAGAG AACTACATTC ATGGTTTGA
 AGTATAAAGA ATAATTTTAA AGACGGTAAA TCTTCTTCTC TTGATGTAAG TACCAAACCT
 1801 AGAGATAAAC CTGAAAAGAA GAGTGGCCTT ATCTTCACTT TATCGATAAG CCAGTTTATT
 TCTCTATTTG GACTTTTCTT CTCACCGGAA TAGAAGTGAA ATAGCTATTC GGTCAAATAA
 1861 TGTTCATTG TGTACATTTT TATATTCTCC TTTTGACATT ATAAGTGTG GCTTTTCTAA
 ACAAAGTAAC ACATGTAAAA ATATAAGAGG AAAACTGTAA TATTGACAAC CGAAAAGATT
 1921 TCTTGTTAAA TATATCTATT TTTACCAAAG GTATTTAATA TTCTTTTTTA TGACAACTTA
 AGAACAAATT ATATAGATAA AAATGGTTTC CATAAATTAT AAGAAAAAAT ACTGTTGAAT
 1981 GATCAACTAT TTTTAGCTTG GTAAATTTTT CTAAACACAA TTGTTATAGC CAGAGGAACA
 CTAGTTGATA AAAATCGAAC CATTAAAAAA GATTGTGTGTT AACAATATCG GTCTCCTTGT
 2041 AAGATGATAT AAAATATTGT TGCTCTGACA AAAATACATG TATTTTCATTC TCGTATGGTG
 TTCTACTATA TTTTATAACA ACGAGACTGT TTTTATGTAC ATAAAGTAAG AGCATACCAC
 2101 CTAGAGTTAG ATTAATCTGC ATTTTAAAAA ACTGAATTGG AATAGAATTG GTAAGTTGCA
 GATCTCAATC TAATTAGACG TAAAATTTTT TGACTTAACC TTATCTTAAC CATTCAACGT
 2161 AAGACTTTTT GAAAATAATT AAATTATCAT ATCTTCCATT CCTGTTATTG GAGATGAAAA
 TTCTGAAAAA CTTTATTATA TTTAATAGTA TAGAAGGTAA GGACAATAAC CTCTACTTTT
 2221 TAAAAAGCAA CTTATGAAAG TAGACATTCA GATCCAGCCA TTAATAACCT ATTCCTTTTT
 ATTTTTCGTT GAATACTTTC ATCTGTAACT CTAGGTCGGT AATGATTGGA TAAGGAAAAA
 2281 TGGGGAAATC TGAGCCTAGC TCAGAAAAAC ATAAAGCACC TTGAAAAAGA CTTGGCAGCT
 ACCCCTTTAG ACTCGGATCG AGTCTTTTTG TATTTCTGTG AACTTTTTCT GAACCGTCGA
 2341 TCCTGATAAA GCGTGCTGTG CTGTGCAGTA GGAACACATC CTATTTATTG TGATGTTGTG
 AGGACTATTT CGCACGACAC GACACGTCAT CCTTGTGTAG GATAAATAAC ACTACAACAC
 2401 GTTTTATTAT CTTAACTCT GTTCCATACA CTTGTATAAA TACATGGATA TTTTATGTA
 CAAAATAATA GAATTTGAGA CAAGGTATGT GAACATATTT ATGTACCTAT AAAAATACAT
 2461 CAGAAGTATG TCTCT
 GTCTTCATAC AGAGA

FIG. 7.

New Sequence + Incyte ESTs

1 ATTTGTTTAA ACCTTGGGAA ACTGGTTCAG GTCCAGGTTT TGCTTTGATC CTTTTCAAAA
 TAAACAAATT TGGAACCCTT TGACCAAGTC CAGGTCCAAA ACGAAACTAG GAAAAGTTTT

61 ACTGGAGACA CAGAAGAGGG CTTCTAGGAA AAAGTTTGG GATGGGATTA TGTGGAAACT
 TGACCTCTGT GTCTTCTCCC GAAGATCCTT TTTCAAAACC CTACCCTAAT ACACCTTTGA

121 ACCCTGCGAT TCTCTGCTGC CAGAGCAGGC TCGGCGCTTC CACCCAGTG CAGCCTTCCC
 TGGGACGCTA AGAGACGACG GTCTCGTCCG AGCCGCGAAG GTGGGGTCAC GTCGGAAGGG

181 CTGGCGGTGG TGAAAGAGAC TCGGGAGTCG CTGCTTCCAA AGTGCCCGCC GTGAGTGAGC
 GACCGCCACC ACTTTCTCTG AGCCCTCAGC GACGAAGGTT TCACGGGCGG CACTCACTCG

+2 Met SerLeuPhe GlyLeuLeu LeuLeuThrSer AlaLeuAl
)-----

241 TCTCACCCCA GTCAGCCAAA TGAGCCTCTT CGGGCTTCTC CTGCTGACAT CTGCCCTGGC
 AGAGTGGGGT CAGTCGGTTT ACTCGGAGAA GCGCGAAGAG GACGACTGTA GACGGGACCG

+2 aGlyGlnArg GlnGlyThrGln AlaGluSer AsnLeuSer SerLysPheGln PheSerSe

301 CGGCCAGAGA CAGGGGACTC AGGCGGAATC CAACCTGAGT AGTAAATTC AGTTTTCCAG
 GCCGTCTCT GTCCCTGAG TCCGCTTAG GTTGACTCA TCATTTAAGG TCAAAGGTC

+2 rAsnLysGlu GlnTyrGlyVal GlnAspPro GlnHisGlu ArgIleIleThr ValSerTh

361 CAACAAGGAA CAGTACGGAG TACAAGATCC TCAGCATGAG AGAATTATTA CTGTGTCTAC
 GTTGTTCCTT GTCATGCCCTC ATGTTCTAGG AGTCGTACTC TCTTAATAAT GACACAGATG

+2 rAsnGlySer IleHisSerPro ArgPhePro HisThrTyr ProArgAsnThr ValLeuVa

421 TAATGGAAGT ATTACAGCC CAAGGTTTCC TCATACTTAT CCAAGAAATA CCGTCTTGGT
 ATTACCTTCA TAAGTGTCGG GTTCCAAAGG AGTATGAATA GGTTCTTTAT GCCAGAACCA

+2 lTrpArgLeu ValAlaValGlu GluAsnVal TrpIleGln LeuThrPheAsp GluArgPh

481 ATGGAGATTA GTAGCAGTAG AGGAAAATGT ATGGATACAA CTTACGTTTG ATGAAAGATT
 TACCTCTAAT CATCGTCATC TCCTTTTACA TACCTATGTT GAATGCAAAC TACTTTCTAA

+2 eGlyLeuGlu AspProGluAsp AspIleCys LysTyrAsp PheValGluVal GluGluPr

541 TGGGCTTGAA GACCCAGAAG ATGACATATG CAAGTATGAT TTTGTAGAAG TTGAGGAACC
 ACCCGAAGTT CTGGGTCTTC TACTGTATAC GTTCATACTA AAACATCTTC AACTCCTTGG

+2 oSerAspGly ThrIleLeuGly ArgTrpCys GlySerGly ThrValProGly LysGlnIl

601 CAGTGATGGA ACTATATTAG GCGCTGGTG TGGTTCTGGT ACTGTACCAG GAAACAGAT
 GTCACCTACCT TGATATAATC CCGCGACCAC ACCAAGACCA TGACATGGTC CTTTGTCTA

+2 eSerLysGly AsnGlnIleArg IleArgPhe ValSerAsp GluTyrPhePro SerGluPr

661 TTCTAAAGGA AATCAAATTA GGATAAGATT TGTATCTGAT GAATATTTTC CTTCTGAACC
 AAGATTTCTT TTAGTTTAAT CCTATTCTAA ACATAGACTA CTTATAAAAG GAAGACTTGG

FIG. 7(CONTINUED 1).

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+2 oGlyPheCys IleHisTyrAsn IleValMet ProGlnPhe ThrGluAlaVal SerProSe
-----
721 AGGGTTCTGC ATCCACTACA ACATTGTCAT GCCACAATTC ACAGAAGCTG TGAGTCCTTC
    TCCCAAGACG TAGGTGATGT TGTAACAGTA CGGTGTTAAG TGTCTTCGAC ACTCAGGAAG

+2 rValLeuPro ProSerAlaLeu ProLeuAsp LeuLeuAsn AsnAlaIleThr AlaPheSe
-----
781 AGTGCTACCC CCTTCAGCTT TGCCACTGGA CCTGCTTAAT AATGCTATAA CTGCCTTTAG
    TCACGATGGG GGAAGTCGAA ACGGTGACCT GGACGAATTA TTACGATATT GACGGAAATC

+2 rThrLeuGlu AspLeuIleArg TyrLeuGlu ProGluArg TrpGlnLeuAsp LeuGluAs
-----
841 TACCTTGGA GACCTTATTC GATATCTTGA ACCAGAGAGA TGGCAGTTGG ACTTAGAAGA
    ATGGAACCTT CTGGAATAAG CTATAGAACT TGGTCTCTCT ACCGTCAACC TGAATCTTCT

+2 pLeuTyrArg ProThrTrpGln LeuLeuGly LysAlaPhe ValPheGlyArg LysSerAr
-----
901 TCTATATAGG CCAACTTGGC AACTTCTTGG CAAGGCTTTT GTTTTTGGAA GAAAATCCAG
    AGATATATCC GGTGGAACCG TTGAAGAACC GTTCCGAAAA CAAAACCTT CTTTATAGTC

+2 gValValAsp LeuAsnLeuLeu ThrGluGlu ValArgLeu TyrSerCysThr ProArgAs
-----
961 AGTGGTGGAT CTGAACCTTC TAACAGAGGA GGTAAGATTA TACAGCTGCA CACCTCGTAA
    TCACCACCTA GACTTGAAG ATTGTCTCTC CCATTCTAAT ATGTCGACGT GTGGAGCATT

+2 nPheSerVal SerIleArgGlu GluLeuLys ArgThrAsp ThrIlePheTrp ProGlyCy
-----
1021 CTCTCAGTG TCCATAAGGG AAGAACTAAA GAGAACCGAT ACCATTTTCT GGCCAGGTTG
    GAAGAGTCAC AGGTATTCCC TTCTTGATTT CTCTTGCTA TGGTAAAAGA CCGGTCCAAC

+2 sLeuLeuVal LysArgCysGly GlyAsnCys AlaCysCys LeuHisAsnCys AsnGluCy
-----
1081 TCTCCTGGTT AAACGCTGTG GTGGGAACTG TGCTGTGTGT CTCCACAATT GCAATGAATG
    AGAGGACCAA TTTGCGACAC CACCCTTGAC ACGGACAACA GAGGTGTAA CGTTACTTAC

+2 sGlnCysVal ProSerLysVal ThrLysLys TyrHisGlu ValLeuGlnLeu ArgProLy
-----
1141 TCAATGTGTC CCAAGCAAAG TTAATAAAAA ATACCACGAG GTCCTTCAGT TGAGACCAAA
    AGTTACACAG GGTTCGTTTC AATGATTTTT TATGGTGCTC CAGGAAGTCA ACTCTGGTTT

+2 sThrGlyVal ArgGlyLeuHis LysSerLeu ThrAspVal AlaLeuGluHis HisGluGl
-----
1201 GACCGGTGTC AGGGGATTGC ACAAATCACT CACCGACGTG GCCCTGGAGC ACCATGAGGA
    CTGGCCACAG TCCCCTAACG TGTTTAGTGA GTGGCTGCAC CGGGACCTCG TGGTACTCCT

+2 uCysAspCys ValCysArgGly SerThrGly Gly
----->
1261 GTGTGACTGT GTGTGCAGAG GGAGCACAGG AGGATAGCCG CATCACCACC AGCAGCTCTT
    CACACTGACA CACACGTCTC CCTCGTGTCC TCCTATCGGC GTAGTGGTGG TCGTCGAGAA

1321 GCCCAGAGCT GTGCAGTGCA GTGGCTGATT CTATTAGAGA ACGTATGCGT TATCTCCATC
    CGGGTCTCGA CACGTCACGT CACCGACTAA GATAATCTCT TGCATACGCA ATAGAGGTAG

1381 CTTAATCTCA GTTGTGTTGCT TCAAGGACCT TTCATCTTCA GGATTTACAG TGCATTCTGA
    GAATTAGAGT CAACAAACGA AGTTCCTGGA AAGTAGAAGT CCTAAATGTC ACGTAAGACT

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FIG. 7 (CONTINUED 2).

1441 AAGAGGAGAC ATCAAACAGA ATTAGGAGTT GTGCAACAGC TCTTTTGAGA GGAGGCCATA
 TTCTCCTCTG TAGTTTGTCT TAATCCTCAA CACGTTGTCT AGAAAACTCT CCTCCGGATT
 1501 AGGACAGGAG AAAAGGTCTT CAATCGTGGA AAGAAAATTA AATGTTGTAT TAAATAGATC
 TCCTGTCCTC TTTTCCAGAA GTTAGCACCT TTCTTTTAAT TTACAACATA ATTTATCTAG
 1561 ACCAGCTAGT TTCAGAGTTA CCATGTACGT ATTCCACTAG CTGGGTCTCTG TATTTTCAGTT
 TGGTCGATCA AAGTCTCAAT GGTACATGCA TAAGGTGATC GACCCAAGAC ATAAAGTCAA
 1621 CTTTCGATAC GGCTTAGGGT AATGTCAGTA CAGGAAAAAA ACTGTGCAAG TGAGCACCTG
 GAAAGCTATG CCGAATCCCA TTACAGTCAT GTCCTTTTTT TGACACGTTT ACTCGTGGAC
 1681 ATTCCGTTGC CTGGCTTAA CTCTAAAGCT CCATGTCCTG GGCCTAAAAT CGTATAAAAT
 TAAGGCAACG GAACCGAATT GAGATTTCTGA GGTACAGGAC CCGGATTTTA GCATATTTTA
 1741 CTGGATTTTT TTTTTTTTTT TTGCGCATAT TCACATATGT AAACCAGAAC ATTCTATGTA
 GACCTAAAAA AAAAAAAAAA AACGCGTATA AGTGATATACA TTTGGTCTTG TAAGATACAT
 1801 CTACAAACCT GGTTTTAA AAGGAACTAT GTTGCTATGA ATTAACTTG TGTCATGCTG
 GATGTTTGGG CCAAAAATTT TTCCTTGATA CAACGATACT TAATTTGAAC ACAGTACGAC
 1861 ATAGGACAGA CTGGATTTTT CATATTTCTT ATTAAAATTT CTGCCATTTA GAAGAAGAGA
 TATCCTGTCT GACCTAAAAA GTATAAGAA TAATTTTAA GACGGTAAAT CTCTTCTCT
 1921 ACTACATTCA TGGTTTGGA GAGATAAAC TGAAAAGAAG AGTGGCCTTA TCTTCACTTT
 TGATGTAAGT ACCAAACCTT CTCTATTGG ACTTTTCTC TCACCGGAAT AGAAGTAAA
 1981 ATCGATAAGT CAGTTTATTT GTTTCATTGT GTACATTTTT ATATTCTCCT TTTGACATTA
 TAGCTATTCA GTCAAATAAA CAAAGTAACA CATGTAAAA TATAAGAGGA AACTGTAAAT
 2041 TAACTGTTGG CTTTCTAAT CTGTGTTAAAT ATATCTATTT TTACCAAAGG TATTTAATAT
 ATTGACAACC GAAAAGATTA GAACAATTTA TATAGATAA AATGGTTTCC ATAAATTATA
 2101 TCTTTTTTAT GACAACTTAG ATCAACTATT TTTAGCTTGG TAAATTTTTT TAAACACAAT
 AGAAAAATA CTGTTGAATC TAGTTGATAA AAATCGAACC ATTTAAAAAG ATTTGTGTTA
 2161 TGTTATAGCC AGAGGAACAA AGATGATATA AAATATTGTT GCTCTGACAA AAATACATGT
 ACAATATCGG TCTCCTTGTT TCTACTATAT TTTATAACAA CGAGACTGTT TTTATGTACA
 2221 ATTCATTTCT CGTATGGTGC TAGAGTTAGA TTAATCTGCA TTTTAAAAAA CTGAATTGGA
 TAAAGTAAGA GCATACCACG ATCTCAATCT AATTAGACGT AAAATTTTTT GACTTAACTT
 2281 ATAGAATTGG TAAGTTGCAA AGACTTTTTG AAAATAATTA AATTATCATA TCTTCCATTC
 TATCTTAACC ATTCAACGTT TCTGAAAAAC TTTTATTAAT TTAATAGTAT AGAAGGTAAG
 2341 CTGTTATTGG AGATGAAAAT AAAAAGCAAC TTATGAAAGT AGACATTCAG ATCCAGCCAT
 GACAATAACC TCTACTTTTA TTTTCGTTG AATACTTTCA TCTGTAAGTC TAGGTGCGTA
 2401 TACTAACCTA TTCCTTTTTT GGGGAAATCT GAGCCTAGCT CAGAAAAACA TAAAGCACCT
 ATGATTGGAT AAGGAAAAA CCCCTTTAGA CTCGGATCGA GTCTTTTGT ATTTCTGTGA
 2461 TGAAAAAGAC TTGGCAGCTT CCTGATAAAG CGTGCTGTGC TGTGCAGTAG GAACACATCC
 ACTTTTTCTG AACCGTCGAA GGACTATTTT GCACGACACG ACACGTCATC CTTGTGTAGG
 2521 TATTTATTGT GATGTTGTGG TTTTATTATC TTAACTCTG TTCCATACAC TTGTATAAAT
 ATAAATAACA CTACAACACC AAAATAATAG AATTTGAGAC AAGGTATGTG AACATATTTA

FIG. 7(CONTINUED 3).

2581 ACATGGATAT TTTTATGTAC AGAAGTATGT CTCTTAACCA GTTCACTTAT TGTACTCTGG
TGTACCTATA AAAATACATG TCTTCATACA GAGAATTGGT CAAGTGAATA ACATGAGACC

2641 CAATTTAAAA GAAAATCAGT AAAATATTTT GCTTGTAATA TGCTTAATAT CGTGCCTAGG
GTTAAATTTT CTTTGTAGTCA TTTTATAAAA CGAACATTTT ACGAATTATA GCACGGATCC

2701 TTATGTGGTG ACTATTTGAA TCAAAAATGT ATTGAATCAT CAAATAAAAAG AATGTGGCTA
AATACACCAC TGATAAACTT AGTTTTTACA TAACTTAGTA GTTTATTTTC TTACACCGAT

2761 TTTTGGGGAG AAAATT
AAAACCCCTC TTTTAA

FIG. 8. Additional oligonucleotides used for amplification of entire coding region

5'-1 TTTGTTTAAACCTTGGGAAACTGG

5'-2 GTCCAGGTTTTGCTTTGATCC

FIG. 9. DNA Sequence Of Clones 4 & 7, Identical Clones Containing The Entire Open Reading Frame

```

1  TTTGTTTAAA CCTTGGGAAA CTGGTTCAGG TCCAGGTTTT GCTTTGATCC TTTTCAAAAA
   AAACAAATTT GGAACCCTTT GACCAAGTCC AGGTCCAAAA CGAAACTAGG AAAAGTTTTT

61  CTGGAGACAC AGAAGAGGGC TCTAGGAAAA AGTTTGGAT GGGATTATGT GGAACTACC
   GACCTCTGTG TCTTCTCCCG AGATCCTTTT TCAAAACCTA CCCTAATACA CCTTTGATGG

121 CTGCGATTCT CTGCTGCCAG AGCAGGCTCG GCGCTTCCAC CCCAGTGCAG CCTTCCCCTG
   GACGCTAAGA GACGACGGTC TCGTCCGAGC CGCGAAGGTG GGGTCACGTC GGAAGGGGAC

181 GCGGTGGTGA AAGAGACTCG GGAGTCGCTG CTTCCAAAGT GCGCGCCGTG AGTGAGCTCT
   CGCCACCACT TTCTCTGAGC CCTCAGCGAC GAAGGTTTCA CGGGCGGCAC TCACTCGAGA

+2                               MetSer LeuPheGly LeuLeuLeu LeuThrSerAla LeuAlaGl
   }-----

241 CACCCAGTC AGCCAAATGA GCCTCTTCGG GCTTCTCCTG CTGACATCTG CCCTGGCCGG
   GTGGGGTCAG TCGGTTTACT CGGAGAAGCC CGAAGAGGAC GACTGTAGAC GGGACCGGCC

+2 yGlnArgGln GlyThrGlnAla GluSerAsn LeuSerSer LysPheGlnPhe SerSerAs
   -----

301 CCAGAGACAG GGGACTCAGG CGGAATCCAA CCTGAGTAGT AAATTCCAGT TTTCCAGCAA
   GGTCTCTGTC CCCTGAGTCC GCCTTAGGTT GGACTCATCA TTTAAGGTCA AAAGGTCGTT

+2 nLysGluGln AsnGlyValGln AspProGln HisGluArg IleIleThrVal SerThrAs
   -----

361 CAAGGAACAG AACGGAGTAC AAGATCCTCA GCATGAGAGA ATTATTACTG TGTCTACTAA
   GTTCCTTGTC TTGCCTCATG TTCTAGGAGT CGTACTCTCT TAATAATGAC ACAGATGATT

+2 nGlySerIle HisSerProArg PheProHis ThrTyrPro ArgAsnThrVal LeuValTr
   -----

421 TGGAAGTATT CACAGCCCAA GGTTTCCTCA TACTTATCCA AGAAATACGG TCTTGGTATG
   ACCTTCATAA GTGTCGGGTT CCAAAGGAGT ATGAATAGGT TCTTTATGCC AGAACCATAC

+2 pArgLeuVal AlaValGluGlu AsnValTrp IleGlnLeu ThrPheAspGlu ArgPheGl
   -----

481 GAGATTAGTA GCAGTAGAGG AAAATGTATG GATACAACTT ACGTTTGATG AAAGATTTGG
   CTCTAATCAT CGTCATCTCC TTTTACATAC CTATGTTGAA TGCAAACTAC TTTCTAAACC

+2 yLeuGluAsp ProGluAspAsp IleCysLys TyrAspPhe ValGluValGlu GluProSe
   -----

541 GCTTGAAGAC CCAGAAGATG ACATATGCAA GTATGATTTT GTAGAAGTTG AGGAACCCAG
   CGAACTTCTG GGTCTTCTAC TGTATACGTT CATACTAAAA CATCTTCAAC TCCTTGGGTC

+2 rAspGlyThr IleLeuGlyArg TrpCysGly SerGlyThr ValProGlyLys GlnIleSe
   -----

601 TGATGGAAGT ATATTAGGGC GCTGGTGTGG TTCTGGTACT GTACCAGGAA AACAGATTTT
   ACTACCTTGA TATAATCCCG CGACCACACC AAGACCATGA CATGGTCCTT TTGTCTAAAG

+2 rLysGlyAsn GlnIleArgIle ArgPheVal SerAspGlu TyrPheProSer GluProGl
   -----

661 TAAAGGAAAT CAAATTAGGA TAAGATTGT ATCTGATGAA TATTTTCCTT CTGAACCAAG
   ATTCCTTTA GTTAAATCCT ATTCTAAACA TAGACTACTT ATAAAGGAA GACTTGGTCC

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FIG. 9(CONTINUED).

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+2 yPheCysIle HisTyrAsnIle ValMetPro GlnPheThr GluAlaValSer ProSerVa
-----
721 GTTCTGCATC CACTACAACA TTGTCATGCC ACAATTCACA GAAGCTGTGA GTCCTTCAGT
    CAAGACGTAG GTGATGTTGT AACAGTACGG TGTTAAGTGT CTTCGACACT CAGGAAGTCA

+2 lLeuProPro SerAlaLeuPro LeuAspLeu LeuAsnAsn AlaIleThrAla PheSerTh
-----
781 GCTACCCCTT TCAGCTTTGC CACTGGACCT GCTTAATAAT GCTATAACTG CCTTTAGTAC
    CGATGGGGGA AGTCGAAACG GTGACCTGGA CGAATTATTA CGATATTGAC GGAAATCATG

+2 rLeuGluAsp LeuIleArgTyr LeuGluPro GluArgTrp GlnLeuAspLeu GluAspLe
-----
841 CTTGGAAGAC CTTATTTCGAT ATCTTGAACC AGAGAGATGG CAGTTGGACT TAGAAGATCT
    GAACCTTCTG GAATAAGCTA TAGAACTTGG TCTCTCTACC GTCAACCTGA ATCTTCTAGA

+2 uTyrArgPro ThrTrpGlnLeu LeuGlyLys AlaPheVal PheGlyArgLys SerArgVa
-----
901 ATATAGGCCA ACTTGGCAAC TTCTTGGCAA GGCTTTTGTT TTTGGAAGAA AATCCAGAGT
    TATATCCGGT TGAACCGTTG AAGAACC GTT CCGAAAACAA AAACCTTCTT TTAGGTCTCA

+2 lValAspLeu AsnLeuLeuThr GluGluVal ArgLeuTyr SerCysThrPro ArgAsnPh
-----
961 GGTGGATCTG AACCTTCTAA CAGAGGAGGT AAGATTATAC AGCTGCACAC CTCGTAACCT
    CCACCTAGAC TTGGAAGATT GTCTCCTCCA TTCTAATATG TCGACGTGTG GAGCATTGAA

+2 eSerValSer IleArgGluGlu LeuLysArg ThrAspThr IlePheTrpPro GlyCysLe
-----
1021 CTCAGTGTCC ATAAGGGAAG AACTAAAGAG AACCGATACC ATTTTCTGGC CAGGTTGTCT
    GAGTCACAGG TATTCCTTC TTGATTCTC TTGGCTATGG TAAAAGACCG GTCCAACAGA

+2 uLeuValLys ArgCysGlyGly AsnCysAla CysCysLeu HisAsnCysAsn GluCysGl
-----
1081 CCTGGTTAAA CGCTGTGGTG GGAAGTGTGC CTGTTGTCTC CACAATTGCA ATGAATGTCA
    GGACCAATTT GCGACACCAC CCTTGACACG GACAACAGAG GTGTTAACGT TACTTACAGT

+2 nCysValPro SerLysValThr LysLysTyr HisGluVal LeuGlnLeuArg ProLysTh
-----
1141 ATGTGTCCCA AGCAAAGTTA CTAAAAATA CCACGAGGTC CTTCAAGTGA GACCAAAGAC
    TACACAGGGT TCGTTTCAAT GATTTTTTAT GGTGCTCCAG GAAGTCAACT CTGGTTTCTG

+2 rGlyValArg GlyLeuHisLys SerLeuThr AspValAla LeuGluHisHis GluGluCy
-----
1201 CGGTGTCAGG GGATTGCACA AATCACTCAC CGACGTGGCC CTGGAGCACC ATGAGGAGTG
    GCCACAGTCC CCTAACGTGT TTAGTGAGTG GCTGCACCGG GACCTCGTGG TACTCCTCAC

+2 sAspCysVal CysArgGlySer ThrGlyGly
----->
1261 TGAAGTGTG TGCAGAGGGA GCACAGGAGG ATAGCCGCAT CACCACCAGC AGCTCTTGCC
    ACTGACACAC ACGTCTCCCT CGTGTCCTCC TATCGGCGTA GTGGTGGTCG TCGAGAACGG

1321 CAGAGCTGTG CAGTGCAGTG GCTGATTCTA TTAGAGAACG TATGCGTTAT CTCCATCCTT
    GTCTCGACAC GTCACGTCAC CGACTAAGAT AATCTCTTGC ATACGCAATA GAGGTAGGAA

1381 AATCTCAGTT GTTTGCTTCA AGGACCTTTC ATCTTCAGGA TTTACAGTGC ATTCTGAAAG
    TTAGAGTCAA CAAACGAAGT TCCTGGAAAG TAGAAGTCCT AAATGTCACG TAAGACTTTC

1441 AGGAGACATC AAACAGAATT AGGAGTTGTG CAA
    TCCTCTGTAG TTTGTCTTAA TCCTCAACAC GTT

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FIG. 10. Predicted Full-length Polypeptide Sequence

1 MSLFGLLLLT SALAGQRQGT QAESNLSSKF QFSSNKEQYG VQDPQHERII
51 TVSTNGSIHS PRFPHTYPRN TVLVWRLVAV EENVWIQLTF DERFGLEDPE
101 DDICKYDFVE VEEPSDGTL GRWCGSGTVP GKQISKGNQI RIRFVSDEYF
151 PSEPGFCIHY NIVMPOFTEA VSPSVLPPSA LPLDLLNNAI TAFSTLEDLI
201 RYLEPERWQL DLEDLYRPTW QLLGKAFVFG RKSrvVDLNL LTEEVRLYSC
251 TPRNFSVSIR EELKRTDTIF WPGCLLVKRC GGNCACCLHN CNECQCVP SK
301 VTKKYHEVLQ LRPKTGVRGL HKSLTDVALE HHEECDCVCR GSTGG

FIG. 11. Alignment of VEGF-X with Other VEGFs

	*	20	*	40	*	
VEGF_HUMAN :	-----		-----			-
PLGF_HUMAN :	-----		-----			-
VEGB_HUMAN :	-----		-----			-
VEGC_HUMAN :	-----		-----			-
VEGD_HUMAN :	-----		-----			-
990126vegx :	MSLFGLLLLTSALAGQRQGTQAESNLSSKFQFSSNKEQNGVQDPQHERII					50
	60	*	80	*	100	
VEGF_HUMAN :	-----		-----			-
PLGF_HUMAN :	-----		-----			-
VEGB_HUMAN :	-----		-----			-
VEGC_HUMAN :	-----		-----			-
VEGD_HUMAN :	-----		-----			-
990126vegx :	TVSTNGSIHSPRFPHTYPRNTVLVWRLVAVEENVWVQLTFDERFGLEDPE					100
	*	120	*	140	*	
VEGF_HUMAN :	-----		-----			-
PLGF_HUMAN :	-----		-----			-
VEGB_HUMAN :	-----		-----			-
VEGC_HUMAN :	-----MHLLGFFSVACSLAAALLPGPREAPAAAA					30
VEGD_HUMAN :	-----MYREWVVNV					10
990126vegx :	DDICKYDFVEV--EESDGTILGRWCGSGTVPKGQISKGNQIRIRFVSDE					148
	160	*	180	*	200	
VEGF_HUMAN :	-----MN					2
PLGF_HUMAN :	-----MP					2
VEGB_HUMAN :	-----					-
VEGC_HUMAN :	AFESGLDLSDAEPDAGEATAYASKDLEEQLRSVSSVDELMTVLYPEYWKM					80
VEGD_HUMAN :	FMMLYVQLVQGSSNEHGPVKRSSQSTLERSEQQIRAASSLEELLRITHSE					60
990126vegx :	YFPSEPGFCIHYNIVMPQFTEAVSPSVLPSPALPLDLLNNAITAFSTLED					198
	*	220	*	240	*	
VEGF_HUMAN :	FLLSWVHWSLALLLYLHHAKWSQAAPMAEGGGQNHHEVVKFMD-VYQRSY					51
PLGF_HUMAN :	VMRLFPCFLQLLAGLALPAVPPQQWALSAGNGSSEVEVVPFQE-VWGRSY					51
VEGB_HUMAN :	---MSPLLRLLLAALLQLAPAPVSQPDAPGHQRKVVSUID-VYTRAT					46
VEGC_HUMAN :	YKQQLRKGGWQHNRQANLNSRTEETIKFAAAHYNTEILKSIDNEWRTQ					130
VEGD_HUMAN :	DWKLRRCRLRLKSFTSMDRSASHRSTRFAATFYDIETLKVIDEEWORTQ					110
990126vegx :	LIRYLEPERWQLDLEDLYRPTWQLLGKAFVFGKSRVVDLNLLEEVRLY					248
	260	*	280	*	300	
VEGF_HUMAN :	CHPIETLVDIFOEYPDEIEYIFKPSVPLMRCCG---CCND--EGLECV					96
PLGF_HUMAN :	CRALERLVDVVS EYPSEVEHMFSPSCVSLLRCTG---CCGD--ENLHCV					96
VEGB_HUMAN :	CQPREVVVPLTVELMGTVAKQLVPSCVTVQRCGG---CCPD--DGLECV					91
VEGC_HUMAN :	CMPREVCIDVGKEFGVATNTFFKPPCVSVYRCGG---CCNS--EGLQCMN					175
VEGD_HUMAN :	CSPRETCVEVASELGKSTNTFFKPPCVSVYRCGG---CCNE--ESLICMN					155
990126vegx :	SCTPRNFSVSIREELKRTDTIFWPGCILVKRCGGNCACCLHNCNECCVP					298

FIG. 11 (CONTINUED).

VEGF_HUMAN	:	TEESNITM	320	QHIGEM	340		
PLGF_HUMAN	:	VETANVTM		PSYVEL			
VEGB_HUMAN	:	TGQHQVRM		SOLGEM			
VEGC_HUMAN	:	TSTSYLSK		PKPVTI			
VEGD_HUMAN	:	TSTSYISK		PELVV			
990126vegx	:	SKVTKKYH		VALEH			
VEGF_HUMAN	:	KSVRGKGK	360		380		400
PLGF_HUMAN	:						
VEGB_HUMAN	:	DSPR					
VEGC_HUMAN	:	SIIRRS					
VEGD_HUMAN	:	IRRSIQI					
990126vegx	:						
VEGF_HUMAN	:		420		440		
PLGF_HUMAN	:						
VEGB_HUMAN	:						
VEGC_HUMAN	:	TDGFHD					
VEGD_HUMAN	:						
990126vegx	:						
VEGF_HUMAN	:		460		480		500
PLGF_HUMAN	:						
VEGB_HUMAN	:						
VEGC_HUMAN	:	LFPSQC					
VEGD_HUMAN	:	HMMFDE					
990126vegx	:						
VEGF_HUMAN	:		520		540		
PLGF_HUMAN	:						
VEGB_HUMAN	:	TCRCR					
VEGC_HUMAN	:	GKKFHH					
VEGD_HUMAN	:	TCSCED					
990126vegx	:						

FIG. 12.

Variant Polypeptide Sequences

	*	20	*	40	*	
FL_seq	:	MSLFGLLLLSALAGORQGTQAESNLSSKFQFSSNKEQNGVQDPQHERII				: 50
clone41	:	MSLFGLLLLSALAGORQGTQAESNLSSKFQFSSNKEQNGVQDPQHERII				: 50
clone20	:	MSLFGLLLLSALAGORQGTQAESNLSSKFQFSSNKEQNGVQDPQHERII				: 50
		60	*	80	*	100
FL_seq	:	TVSTNGSIHSPRFPHITYPRNTVLVWRLVAVEENVWIQLTFDERFGLEDPE				: 100
clone41	:	TVSTNGSIHSPRFPHITYPRNTVLVWRLVAVEENVWIQLTFDERFGLEDPE				: 100
clone20	:	TVSTNGSIHSPRFPHITYPRNTVLVWRLVAVEENVWIQLTFDERFGLEDPE				: 100
		*	120	*	140	*
FL_seq	:	DDICKYDFVEVEEPSDGTILGRWCGSGTVPKGQISKGNQIRIRFVSDEYF				: 150
clone41	:	DDICKYDFVEVEEPSDGTILGRWCGSGTVPKGQISKGNQIRIRFVSDEYF				: 150
clone20	:	DDICKYDFVEVEEPSDGTILGRWCGSGTVPKGQISKGNQIRIRFVSDEYF				: 150
		160	*	180	*	200
FL_seq	:	PSEPGFCIHYNIVMPQFTEAVSPSVLPFSALEPLDLLNNAITAFSTLEDLI				: 200
clone41	:	PSEPSNRGGKIIQLHTS-----				: 167
clone20	:	PSEPGFCIHYNIVMPQFTEAVSPSVLPFSALEPLDLLNNAITAFSTLEDLI				: 200
		*	220	*	240	*
FL_seq	:	RYLEPERWQLDLEDLYRPTWQLLGKAFVFGRKSRVVDLNLLEEVRLYSC				: 250
clone41	:	-----				: -
clone20	:	RYLEPERWQLDLEDLYRPTWQLLGKAFVFGRKSRVVDLNLLE-----				: 243
		260	*	280	*	300
FL_seq	:	TPRNFSVSIREELEKRTDTIFWPGCLLVKRCGGNCACCLHNCNECQCVPSK				: 300
clone41	:	-----				: -
clone20	:	-----				: -
		*	320	*	340	
FL_seq	:	VTKKYHEVLQLRPKTGVRGLHKS LTDVALEHHEECDVCVRGSTGG				: 345
clone41	:	-----				: -
clone20	:	-----EVLQLRPKTGVRGLHKS LTDVALEHHEECDVCVRGSTGG				: 282

FIG. 13. Primers for Expression of VEGF-X*E.coli expression of domain-*

vegX-6 AATTGGATCCGAGAGTGGTGGATCTGAACC
vegX-7 AATTGGATCCGGGAAGAAAATCCAGAGTGG
vegX-8 GGTTGAATTCATTATTTTTAGTAACTTTGCTTGGGACAC
vegX-9 AATTGAATTCATTATCCTCCTGTGCTCCCTC

Baculovirus/insect cell expression of full-length protein-

vegbac1
AATTGGATCCGGAGTCTCACCATCACCACCATCATGAATCCAACCTGAGTAGTAAATTC
C
vegbac2 AATTGAATTCGCTATCCTCCTGTGCTCCCTCTGC

FIG. 14.

>3993180H1 LUNGNON03 INCYTE
CACAAATCACTCACCGACGTGGCCCTGGAGCACCATGAGGNGTGTGACTGTGTGTGCAGAGGGAGCACAGGAGGATAGCC
GCATCACCACCAGCAGCTCTTGCCACAGAGCTGTGCAGTGCAGTGGCTGATTCTATTAGAGAACGTATGCGTTATCTCCAT
CCTTAATCTCAGTTGTTGCTTCAAGGACCTTTCATCTTCAGGATTTACAGTGCATTCTGAAAGAGGAGACATCAAACAG
AATTAGGAGTTGTGCAACAGCTCTTTGAGAGGAGGCTAAAGGACAGGAGAAAGGTCTT
>3510192H1 CONCN01 INCYTE
TGCAGTGCAGTGGCTGATTCTATTAGAGAACGTATGCGTTATCTCCATCCTTAATCTCAGTTGTTGCTTCAAGGACCTT
TCATCTTCAGGATTTACAGTGCATTCTGAAAGAGGAGACATCAAACAGGATTTAGGAGTTGTGCAACAGCTCTTTGAGAG
GAGGCCCTAAAGGACAGGAGAAAAGGTCTTCAATCGTGGAAGAAAATTAATGTTGTATTAAATAGATCACCAGCTAGTT
TCAGAGTTACCATGTACGTATTCCACTAGCTGGGTTCTGTATTT
>2559870H1 ADRETUT01 INCYTE
CACCAGGTCTTTCAGTTGAGACCAAAGACCGGTGTGAGGGGATTGCACAAATCACTCACCGACGTGGCCCTGGAGCACCA
TGAGGAGTGTGACTGTGTGTCAGAGGGAGCACAGGGGATAGCCGCATCACCACCAGCAGCTCTTGCCACAGAGCTGTGC
AGTGCAGTGGCTGATTCTATTAGAGAACGTATGCGTTATCTCCATCCTTAATCTCAGTTGTTGCTTCAAGGACCTTTCA
TCTTCAGGATTTACAGTGCATTCTGAAAGAGGAGA
>3979767H1 LUNGTUT08 INCYTE
GGAGGATAGCCGCATCACCACCAGCAGCTCTTGCCACAGAGCTGTGCAGTGCAGTGGCTGATTCTATTAGAGAACGTATGC
GTTATCTCCATCCTTAATCTCAGTTGTTGCTTCAAGGACCTTTTCATCTTCAGGATTTACAGTGCATTCTGAAAGAGGAG
ACATCAAACAGAATTAGGAGTTGTGCAACAGCTCTTTTGAGAGGAGGCTAAAGGACAGGAGAAAAGGTCTTCAATCGTG
GAAAGAAATTAATGTTGTATTAAATAGACACCGCT
>3980011H1 LUNGTUT08 INCYTE
GGAGGATAGCCGCATCACCACCAGCAGCTCTTGCCACAGAGCTGTGCAGTGCAGTGGCTGATTCTATTAGAGAACGTATGC
GTTATCTCCATCCTTAATCTCAGTTGTTGCTTCAAGGACCTTTTCATCTTCAGGATTTACATGCATTCTGAAAGAGGAGA
CATCAAACAGAATTAGGAGTTGTGCAACAGCTCTTTTGAGAGGAGGCTAAAGGACAGGAGAAAAGGTCTTCAATCGTGG
AAAGAAAATTAATGTTGTATTAAATAGATCACCA
>4825396H1 BLADDIT01 INCYTE
GAGAACCGATACCATTTTCTGGCCAGGTTGTCTCTGGTTAAACGCTGTGGTGGAACTGTGCCTGTTGTCTCCACAATT
GCAATGAATGTCAATGTGTCCCAAGCAAAGTTACTAAAAAATACCACGAGGTCCTTCAGTTGAGACCAAAGACCGGTGTG
AGGGGATTGCACAAATCACTCACCGACGTGGCCCTGGAGCACCATGAGGAGTGTGACTGTGTGTGCAGAGGGAGCACAGG
AGGATAGCCGCATCACCACCA
>3073703H1 BONEUNT01 INCYTE
AGAAAATCCAGAGTGGTGGATCTGAACCTTCTAACAGAGGAGGTAAGATTATACAGCTGCACACCTCGTAACTTCTCAGT
GTCCATAAGGGAAGAACTAAAGAGAACCGATACCATTTCTGGCCAGGTTGTCTCTGGTTAAACGCTGTGGTGGGAACF
GTGCCTGTTGTCTCCACAATTGCAATGAATGTCAATGTGTGTCCTCAAGCAAAGTTACTAAAAAATACCACGAGGTCCTTCAG
TTGAGACCAAAGACCGGTGTGTCAGGGGATTGCACAAATCA
>1302516H1 PLACNOT02 INCYTE
AGGAAATCAAATTAGGATAAGATTTGTATCTGATGAATATTTCTCTTGAACCTTCTAACAGAGGAGGTAAGATTATAC
AGCTGCACACCTCGTAACTTCTCAGTGTCCATAAGGGAAGAACTAAAGAGAACCGATACCATTTTCTGGCCAGGTTGTCT
CCTGGTTAAACGCTGTGGTGGGAACGTGTGCCTGTTGTCTCCACAATTGCAATGAATGTCAATGTGTCCCAAGCAAAGTT
ACTAAAAAATACCACGAGGTCC
>3684109H1 HEANOT01 INCYTE
ATTTTCATCTTCAGGATTTACAGTGCATTCTGAAANAGGAGAAATCAAACANAATTAGGAGTTGTGCAACAGCTCTTTTGA
GAGGAGGCCCTAAAGGACAGGAGAAAAGGTCTTCAATCGTGGAANAAAATTAATGTTGTATTAAATAGATCACCAGCTA
GTTTCAGAGTTACCATGTACGTATTCCACTAGCTGGGTTCTGTATTTCAGTTCTTTCGATACGGCTTAGGGTAATGTGAG
TACAGGAAAAAACTGTGCAAGTGAGCACCTGATTCCGTTGCCTTGCTT
>4713188H1 BRAIHCT01 INCYTE
CAAAGTTACTAAAAAATACCACGAGGTCTTTCAGTTGAGACCAAAGACCGGTGTGAGGGGATTGCACAAATCACTCACCG
ACGTGGCCCTGGAGCACCATGAGGAGTGTGACTGTGTGTGCAGAGGGAGCACAGGAGGATAGCCGCATCACCACCAGCAG
CTCTTGCCACAGAGCTGTGCAGTGCAGTGGCTGATTCTATTAGAGAACGTATGCGTTATCTCCATCCTTAATCTCAGTTG
TTGCT
>458823H1 KERANOT01 INCYTE
ANGAGTTGCCACAGAGCTGTGCAGTGCAGTGGCTGATTCTATTAGAGAACGTATGCGTTATCTCCATCCTTAATCTCAGT
GTTTGNTTCAAGGACCTTTCATCTTCAGGATTTACAGTGCATTCTGAAAGAGGAGACATCAAACAGAATTAGGAGTTGTG
CAACAGCTCTTTTGAGAGGAGGCCCTAAAGGNCAGGAGAAAAGGTCTTCAATCGTGGAAGAAAATTAATGTTGTATTAA
ATAGATC
>1303909H1 PLACNOT02 INCYTE
AGGAAATCAAATTAGGATAAGATTTGTATCTGATGAATATTTCTCTTGAACCTTCTAACAGAGGAGGTAAGATTATAC
AGCTGCACACCTCGTAACTTCTCAGTGTCCATAAGGGAAGAACTAAAGAGAACCGATACCATTTTCTGGCCAGGTTGTCT
CCTGGTTAAACGCTGTGGTGGGAACGTGTGCCTGTTGTCTCCACAATTGCAATGAATGTCAATGTGTCCCAAG

FIG. 14 (CONTINUED).

>2739211H1 OVARNOT09 INCYTE
GTGCATTCTGAAAGAGGAGACATCAAACAGAATTAGGAGTTGTGCAACAGCTCTTTTGAGAGGAGGCCCTAAAGGACAGGA
GAAAAGGCTTCAATCGTGGAAAGAAAATTAAATGTTGTATTAAATAGATCACCAGCTAGTTTCAGAGTTACCATGTACG
TATTCCACTAGCTGGGTTCTGTATTTTCAGTTCTTTTCGATACGGCTTAGGGTAATGTCAGTACAGGAAAAAACTGTGCAA
GTGAGCACCTGAT

>3325591H1 PTHYNOT03 INCYTE
TGCAACAGCTCTTTTGAGAGGAGGCCCTAAAGGACAGGAGAAAAGGCTTCAATCGTGGAAAGAAAATTAAATGTTGTATT
AAATAGATCACCAGCTAGTTTCAGAGTTACCATGTACGTATTCCACTAGCTGGGTTCTGTATTTTCAGTTCTTTTCGATACG
GCTTAGGGTAATGTCAGTACAGGAAAAAACTGTGCAAGTGAGCACCTGATTCCGTTGCCTTGCTTAACCCCTAAAGCNCC
ATGTCNNGGGCNAAAANCGAAAAAT

>3733565H1 SMCCNOS01 INCYTE
CCTTAATCTCAGTTGTTTGCTTCAAGGACCTTTCATCTTCAGGATTTACAGTGCATTCTGNAAGANGAGACATCAAACAG
AATTAGGNGTTGTGCAAAAGCTCTTTTGAGAGGAGGCCCTAAAGGACAGGAGAAAAGGCTCNCAATCGTGGAAAGNAAATT
AAATGTTGTATNAAATNGATCACCAGCTAGTTTCAGAGTTACCATGTACGTATTCCACTAGCTGGGNCNGTATTTCAGTCT
TTCGGAACGGCTTAGGGTAATGTCAGTACAGGAAAAAACTGTGCAGTGAG

>3554223H1 SYNONOT01 INCYTE
ATTAAATAGATCACCAGCTAGTTTCAGAGTTACCATGTACGTATTCCACTAGCTGGGTTCTGTATTTTCAGTTCTTTTCGAT
ACGGCTTAGGGTAATGTCAGTACAGGAAAAAACTGTGCAAGTGAGCACCTGATTCCGTTGCCTTGGCTTAACCTCTAAAG
CTCCATGTCTGGGCCCTAAATCGTATAAAATCTGGATTTTTTTTTTTTTTTTGGCATATTACATATGTAAACCAGN
ACATTCTATGTACNACAAACCTGGTTTTTAAAAAGGAAC

>4507477H1 OVARTDT01 INCYTE
GGCTAGTTTCAGAGTTACCATGTACGTATTCCACTAGCTGGGTTCTGTATTTTCAGTTCTTTTCGATACGGCTTAGGGTAAT
GTCAGTACAGGAAAAAACTGTGCAAGTGAGCACCTGATTCCGTTGCCTTGCTTAACCTCTAAAGCTCCATGTCTCTGGGCC
TAAATCGTATAAAATCTGGA

>4163378H1 BRSTNOT32 INCYTE
AATAGATCACCAGCTAGTTTCAGAGTTACCATGTACGTATTCCACTAGCTGGGNTCTGTATTTTCAGTTCTTTTCGATACG
GCTTAGGGTAATGTCAGTACAGGAAAAAGCTGTGCAAGTGAGCACCTGATTCCGTTGCCTTGCTTAACCTCTAAAGCTCC
ATGTCTGGGCCCTAAATCGTATA

FIG. 15.

>2054675H1 BEPINOT01 INCYTE
AAAGGAACATATGTTGCTATGAATTAACCTTGTGTCGTGCTGATAGGACAGACTGGATTTTTCATATTTCTTATTAATTA
TCTGCCATTTAGAGAAGAGAACTACATTCATGGTTTGAAGAGATAAACCTGAAAAGAAGAGTGGCCTTATCTTCACTT
TATCGATAAGTCAGTTTATTTGTTTCATTGTGTACATTTTTATATTCCTCTTTTGACATTATAACTGTTGGCTTTTCTAA
TCTTGTTAAATATATCTATTTTACCAAAGGTATTTAATATTCCTTTTTTA
>3993180H1 LUNGNON03 INCYTE
CACAAATCACTCACCGACGTGGCCCTGGAGCACCATGAGGNGTGTGACTGTGTGTGTCAGAGGGAGCACAGGAGGATAGCC
GCATCACCACCAGCAGCTCTTGCCAGAGCTGTGCAGTGCAGTGGCTGATTCTATTAGAGAACGTATGCGTTATCTCCAT
CCTTAATCTCAGTTGTTTGTCTCAAGGACCTTTTCATCTTCAGGATTTACAGTGCATTCTGAAAAGAGGAGACATCAAACAG
AATTAGGAGTTGTGCAACAGCTCTTTTGAGAGGAGGCTAAAGGACAGGAGAAAGGTCCT
>3510192H1 CONCNOT01 INCYTE
TGCAGTGCAGTGGCTGATTCTATTAGAGAACGTATGCGTTATCTCCATCCTTAATCTCAGTTGTTTGTCTCAAGGACCTT
TCATCTTCAGGATTTACAGTGCATTCTGAAAAGAGGAGACATCAAACAGAATTAGGAGTTGTGCAACAGCTCTTTTGAGAG
GAGGCCATAAGGACAGGAGAAAAGGCTTCAATCGTGGAAAGAAAATTAATGTTGTATTAAATAGATCACCAGCTAGTT
TCAGAGTTACCATGTACGTATTCCTAGCTGCGTTCTGTATTT
>4164633H1 BRSTNOT32 INCYTE
CTTGTTAAATATATCTATTTTTACCAAAGGTATTTAATATCTTTANTTATGACAACTTAGATCAACTATTTTTAGCTTG
GTAAATTTTCTAAACACAATTGTTATAGCCAGAGGAACAAAGATGATATAAAATATTGTTGCTCTGACAAAAATACATG
TATTTCAATCTCGTATGGTGTAGAGTTAGATTAATCTGCATTTTAAAAAACTGAATTGGAATAGAATTGGTAAGTTGCA
AAGACTTTTGAANAATAATTAATATCATATCTTCCATCTCTGTTATTGGGGGAGAAAAAT
>2559870H1 ADRETUT01 INCYTE
CACGAGGTCTTTCAGTTGAGACCAAGACCGGTGTGAGGGGATTGCACAAATCACTCACCGACGTGGCCCTGGAGCACCA
TGAGGAGTGTGACTGTGTGTGTCAGAGGGAGCACAGGGGATAGCCGCATCACCACCAGCAGCTCTTGCCAGAGCTGTGC
AGTGCAGTGGCTGATTCTATTAGAGAACGTATGCGTTATCTCCATCCTTAATCTCAGTTGTTTGTCTCAAGGACCTTTCA
TCTTCAGGATTTACAGTGCATTCTGAAAGAGGAGA
>3817470H1 BONSTUT01 INCYTE
TTAAAAAGGAACATATGTTGCTATGAATTAACCTTGTGTATGCTGATAGGACAGACTGGATTTTTCATATTTCTTATTA
AATTTCTGCCATTTAGAGAAGAGAACTACATTCATGGTTTGAAGAGATAAACCTGAAAAGAAGAGTGGCCTTATCTTC
ACTTTATCGATAAGTCAGTTTATTTGTTTCATTGTGTACATTTTTATATTCCTTTTGACATTATAACTGTTGGCTTTC
TAATCTGTTAAATATATCTATTTTTACCAAAGGTATTTAATATTCCTTT
>3979767H1 LUNGTUT08 INCYTE
GGAGGATAGCCGCATCACCACCAGCAGCTCTTGCCAGAGCTGTGCAGTGCAGTGGCTGATTCTATTAGAGAACGTATGC
GTTATCTCCATCCTTAATCTCAGTTGTTTGTCTTCAAGGACCTTTTCATCTTCAGGATTTACAGTGCATTCTGAAAGAGGAG
ACATCAAACAGAATTAGGAGTTGTGCAACAGCTCTTTTGAGAGGAGGCCTAAAGGACAGGAGAAAAGGCTTCAATCGTG
GAAAGAAATTAATGTTGTTGATTAAATAGACACAGCT
>3980011H1 LUNGTUT08 INCYTE
GGAGGATAGCCGCATCACCACCAGCAGCTCTTGCCAGAGCTGTGCAGTGCAGTGGCTGATTCTATTAGAGAACGTATGC
GTTATCTCCATCCTTAATCTCAGTTGTTTGTCTTCAAGGACCTTTTCATCTTCAGGATTTACATGCATTCTGAAAGAGGAGA
CATCAAACAGAATTAGGAGTTGTGCAACAGCTCTTTTGAGAGGAGGCCTAAAGGACAGGAGAAAAGGCTTCAATCGTGG
AAGAAAAATTAATGTTGTATTAAATAGATCACCA
>4825396H1 BLADDIT01 INCYTE
GAGAACCAGATACCATTTTCTGGCCAGGTTGTCTCTGGTTAAACGCTGTGGTGGGAACGTGTGCCTGTTGTCTCCACAATT
GCAATGAATGTCAATGTGTCCCAAGCAAAGTTACTAAAAAATACCACGAGGTCCTTCAGTTGAGACCAAAGACCGGTGTC
AGGGGATTGCACAAATCACTCACCGACGTGGCCCTGGAGCACCATGAGGAGTGTGACTGTGTGTGTCAGAGGGAGCACAGG
AGGATAGCCGCATCACCA
>3073703H1 BONEUNT01 INCYTE
AGAAAATCCAGAGTGGTGGATCTGAACCTTCTAACAGAGGAGGTAAGATTATACAGCTGCACACCTCGTAACCTTCTCAGT
GTCCATAAGGGAAGAACTAAAGAGAACCAGATACCATTTTCTGGCCAGGTTGTCTCTGGTTAAACGCTGTGGTGGGAAC
GTGCCTGTTGTCTCCACAATTGCAATGAATGTCAATGTGTCCCAAGCAAAGTTACTAAAAAATACCACGAGGTCCTTCAG
TTGAGACCAAAGACCGGTGTGACGGGATTGCAAGAAATCA
>862169H1 BRAITUT03 INCYTE
AGATGATATAAAATATTGTTGCTCTGACAAAAATACATGTATTTTCATTCTCGTATGGTGTAGAGTTAGATTAACTGCA
TTTTAAAAAACTGAATTGGAATAGAATTGGTAAGTTGCAAGACTTTTTGAAAATAATTAATATCATATCTTCCATTC
CTGTTATTGGAGATGAAAATAAAAAGCAACTTATGAAAGTAGACATTCAGATCCAGCCATTACTAACCTATTCTTTT
GGGGAATCTGAGCCTAGC
>4201385H1 BRAITUT29 INCYTE
TTTTTAAAAAGGAACATATGTTGCTATGAATTAACCTTGTGTCGTGCTGATAGGACAGACTGGATTTTTCATATTTCTTAT
TAAATTTCTGCCATTTAGAGAAGAGAACTACATTCATGGTTTGAAGAGATAAACCTGAAAAGAAGAGTGGCCTATCT
TCATTTATCGATAAGTCAGTTTATTTGTTTCATTGTGTACATTTTTATATCTCTCTTTGACATATAACTGTTGGCTTTT

FIG. 15 (CONTINUED 1).

CTAATCTGTTAAATATATCTATTTTTTACCAAAGGTATTTAATAT
>1302516H1 PLACNOT02 INCYTE
AGGAAATCAAATTAGGATAAGATTTGTATCTGATGAATATTTTCCTTCTGAACCTTCTAACAGAGGAGGTAAGATTATAC
AGCTGCACACCTCGTAACTTCTCAGTGTCCATAAGGGAAGAACTAAAGAGAACCGATACCATTCTTCTGGCCAGGTTGTCT
CCTGGTTAAACGCTGTGGTGGGAAGTGTGCCTGTTGTCTCCACAATTGCAATGAATGTCAATGTGTCCCAAGCAAAGTT
ACTAAAAAATACCACGAGGTCC
>3684109H1 HEANOT01 INCYTE
ATTTTCATCTTCAGGATTTACAGTGCATTCTGAAANAGGAGAAATCAAACANAATTAGGAGTTGTGCAACAGCTCTTTTGA
GAGGAGGCCATAAGGACAGGAGAAAAGGCTTCAATCGTGGAAANAAAAATTAATGTTGTATTAAATAGATCACCAGCTA
GTTTCAGAGTTACCATGTACGTATTCCTAGCTGGGTTCTGTATTTTCAGTTCTTTTCGATACGGCTTAGGGTAATGTCTAG
TACAGGAAAAAACTGTGCAAGTGAGCACCTGATTCCGTTGCCTTGCTT
>2549720H1 LUNGTUT06 INCYTE
TTAGCTTGGNAAATTTTTCTAAACACAATTGTTATAGCCAGAGGAACAAAGATGATATAAAATATTGTTGCTCTGACAAA
AATACATGTATTTTCATTCTCGTATGGTGTAGAGTTAGATTAATCTGCATTTTAAAAAAGTGAATTGGAATAGAATTGGT
AAGTTGCAAGAGACTTTTGGAAATAATTAAATTATCATATCTTCCATTCTGTTATTGGAGATGAAAAAATAAAGCAACT
TATGANAGTAG
>877279H1 LUNGAST01 INCYTE
CTTTTTTATGACAACTTAGATCAACTATTTTTTAGCTTGGTAAATTTTTCTAAACACAATTGTTATAGCCAGAGGAACAAA
GATGATATAAAATATTGTTGCTCTGACAAAAATACATGTATTTTCATTCTCGTATGGTGTAGAGTTAGATTAATCTGCAT
TTTAAAAAAGTGAATTGGAATAGAATTGGTAAGTTGCAAGGCTTTTGGAAATAATTAAATTATCATATCTTCCATTCC
TGTTATTGGNGG
>4713188H1 BRAIHCT01 INCYTE
CAAAGTTACTAAAAAATACCACGAGGTCTTTCAGTTGAGACCAAAGACCGGTGTCAGGGGATTGCACAAATCACTCACCG
ACGTGGCCCTGGAGCACCATGAGGAGTGTGACTGTGTGTGTCAGAGGGAGCACAGGAGGATAGCCGCATCACCACCAGCAG
CTCTTGCCCCAGAGCTGTGCAGTGCAGTGGCTGATTCTATTAGAGAACGTATGCGTTATCTCCATCCTTAATCTCAGTTGT
TTGCT
>2171082H1 ENDCNOT03 INCYTE
AGATAAAGCTGAAAAGAAGAGTGGCCTTATCTTCACTTTATCGATAAGTCAGTTTATTTGTTTCATTGTGTACATTTTA
TATCTCCTTTTGACATTATACTGTGGCTTTTCTAATCTGTTAAATATATCTATTTTTTACCAAAGGTATTTAATATT
CTTTTTTATGACAACTTAGATCAACTATTTTTAGCTTGGTAAATTTTTCTAAACACAATTGTTATAGCCAGAGGAACAAA
GATGA
>875860H1 LUNGAST01 INCYTE
CTGGATTTTTTCATATTTCTTATTAATTTCTGCCATTTAGAAGAAGAGAAGTACATTCATGGTTTGGGAAGAGATAAACC
TGAAAAGAAGAGTGGCCTTATCTTCACTTTATCGATAAGTCAGTTTATTTGTTTCATTGTGTACATTTTATATTCTCCT
TTTGACATTATAACTGTTGGCTTTTCTAATCTGTTAAATATATCTATTTTTTACCAAAGGTATTTAATATTCTTTTTAT
GAC
>706168H1 SYNORAT04 INCYTE
GCTCATATTCACATATGTAAACCAGAACATTCTATGTACTACAAACCTGGTTTTTAAAAAGGANCTATGTTGCTATGAAT
TAACTTGTGTCTGTGCTGATAGGACAGACTGGATTTTTCATATTTCTTATTAATAATTTCTGCCATTTAGAAGAAGAGAAC
TACATTTCATGGTTTGGGAAGAGATAAACCTGAAAAGAAGAGTGGCCTTATCTTCANTTTATCGATAAGTCAGTTTATTTGT
TTCA
>458823H1 KERANOT01 INCYTE
ANGAGTTGCCCAGAGCTGTGCAGTGCAGTGGCTGATTCTATTAGAGAACGTATGCGTTATCTCCATCCTTAATCTCAGTT
GTTTGNTTCAAGGACCTTTCATCTTCAAGATTTACAGTGCATTCTGAAAGAGGAGACATCAAACAGAATTAGGAGTTGTG
CAACAGCTCTTTTGAGAGGAGGCCATAAGGNCAGGAGAAAAGGTCTTCAATCGTGGAAAGAAAATTAATGTTGTATTAA
ATAGATC
>538436H1 LNODNOT02 INCYTE
AAAGATGATATAAAATATGTTGCTCTGACAAAAATACATGTATTTTCATTCTCGTATGGTGTAGAGTTAGATTAATCTG
CATTTTAAAAAAGTGAATTGGAATAGAATTGGTAAGTTGCAAGACTTTTGGAAATAATTAAATTATCATATCTTCCAT
TCCTGTTATTGGAGATGAAAATAAAAAGCACTTATGAAAGTAGACATTCAGATCCAGCCATTACTAACCTAT
>1303909H1 PLACNOT02 INCYTE
AGGAAATCAAATTAGGATAAGATTTGTATCTGATGAATATTTTCCTTCTGAACCTTCTAACAGAGGAGGTAAGATTATAC
AGCTGCACACCTCGTAACTTCTCAGTGTCCATAAGGGAAGAACTAAAGAGAACCGATACCATTCTTCTGGCCAGGTTGTCT
CCTGGTTAAACGCTGTGGTGGGAAGTGTGCCTGTTGTCTCCACAATTGCAATGAATGTCAATGTGTCCCAAG
>2739211H1 OVARNOT09 INCYTE
GTGCATTCTGAAAGAGGAGACATCAAACAGAAATAGGAGTTGTGCAACAGCTCTTTTGGAGAGGAGGCCATAAGGACAGGA
GAAAAGGTCTTCAATCGTGGAAAGAAAATTAATGTTGTATTAAATAGATCACCAGCTAGTTTCAGAGTTACCATGTACG
TATTCACACTAGCTGGGTTCTGTATTTTCAGTTCTTTTCGATACGGCTTAGGGTAATGTGAGTACAGGAAAAAACTGTGCAA
GTGAGCACCTGAT

[illegible]

SUBSTITUTE SHEET (RULE 26)

FIG. 16.

VEGFE1	AAAATGTATGGATACAACTTAC	22
VEGFE2	GTTTGATGAAAGATTGGGCTTG	23
VEGFE3	TTTCTAAAGGAAATCAAATTAG	22
VEGFE4	GATAAGATTTGTATCTGATG	20
VEGFE5	GATGTCTCCTCTTTCAG	17
VEGFE6	GCACAACTCCTAATTCTG	18
VEGFE7	AGCACCTGATTCCGTTGC	19
VEGFE8	TAGTACATAGAATGTTCTGG	20
VEGFE9	AAGAGACATACTTCTGTAC	19
VEGFE10	CCAGGTACAATAAGTGAAGT	21

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FIG. 17.

```

      +3
N L L T E E V R L Y
                                     M N I F L L
                                     ]-----

1  AGGAAATCAA ATTAGGATAA GATTTGTATC TGATGAATAT TTTCCTTCTG
AACCTTCTAA CAGAGGAGGT AAGATTATAC
   TCCTTTAGTT TAATCCTATT CTAAACATAG ACTACTTATA AAAGGAAGAC
TTGGAAGATT GTCTCCTCCA TTCTAATATG

      +3 S C T P R N F S V S I R E E L K R
T D T I F W P G C L
                                     }-----

81 AGCTGCACAC CTCGTAACCTT CTCAGTGTCC ATAAGGGAAG AACTAAAGAG
AACCGATACC ATTTTCTGGC CAGGTTGTCT
   TCGACGTGTG GAGCATTGAA GAGTCACAGG TATTCCCTTC TTGATTCTC
TTGGCTATGG TAAAAGACCG GTCCAACAGA
      -2
                                     <-----

      +3 L V K R C G G N C A C C L H N C N
E C Q C V P S K V
                                     }-----

161 CCTGGTTAAA CGCTGTGGTG GGAAGTGTGC CTGTTGTCTC CACAATTGCA
ATGAATGTCA ATGTGTCCCA AGCAAAGTTA
   GGACCAATTT GCGACACCAC CCTTGACAGG GACAACAGAG GTGTTAACGT
TACTTACAGT TACACAGGGT TCGTTTCAAT
      -2
                                     }-----

      +3 T K K Y H E V L Q L R P K T G V R
G L H K S L T D V A
                                     }-----

      +1
D C T N H S P T W P
                                     V S G
                                     ]-----

241 CTAAAAAATA CCACGAGGTC CTTCAGTTGA GACCAAAGAC CGGTGTCAGG
GGATTGCACA AATCACTCAC CGACGTGGCC
   GATTTTTTAT GGTGCTCCAG GAAGTCAACT CTGGTTTCTG GCCACAGTCC
CCTAACGTGT TTAGTGAGTG GCTGCACCGG
      -2
-----[

      +3 L E H H E E C D C V C R G S T G G

```

FIG. 17 (CONTINUED).

```

>
      +2
I A A S P P A A L A
      +1 W S T M R S V T V C A E G A Q E D
S R I T T S S S C

-----
321 CTGGAGCACC ATGAGGAGTG TGA CTGTGTG TGCAGAGGGA GCACAGGAGG
    ATAGCCGCAT CACCACCAGC AGCTCTTGCC
      GACCTCGTGG TACTCCTCAC ACTGACACAC ACGTCTCCCT CGTGTCTCTCC
    TATCGGCGTA GTGGTGGTCG TCGAGAACGG

      +2 Q S C A V Q W L I L L E N V C V I
S I L N L S C L L Q

-----
      +1 P E L C S A V A D S I R E R M R Y
L H P

----->
401 CAGAGCTGTG CAGTGCAGTG GCTGATTCTA TTAGAGAACG TATGCGTTAT
    CTCCATCCTT AATCTCAGTT GTTGTCTTCA
      GTCTCGACAC GTCACGTCAC CGACTAAGAT AATCTCTTGC ATACGCAATA
    GAGGTAGGAA TTAGAGTCAA CAAACGAAGT

      +2 G P F I F R I Y S A F
----->
481 AGGACCTTTC ATCTTCAGGA TTTACAGTGC ATTCTGAAAG AGGAGACATC
    AAACAGAATT AGGAGTTGTG CAACAGCTCT
      TCCTGGAAAG TAGAAGTCCT AAATGTCACG TAAGACTTTC TCCTCTGTAG
    TTTGTCTTAA TCCTCAACAC GTTGTCTGAGA

561 TTTGAGAGGA GGCCTAAAGG ACAGGAGAAA AGGTCTTCAA TCGTGGAAAG
    AAAATTAAAT GTTGTATTAA ATAGATCACC
      AAACCTCTCT CCGGATTTCC TGTCTCTTTT TCCAGAAGTT AGCACCTTTC
    TTTTAATTTA CAACATAATT TATCTAGTGG

641 AGCTAGTTTC AGAGTTACCA TGTACGTATT CCACTAGCTG GGTCTGTAT
    TTCAGTTCTT TCGATACGGC TTAGGGTAAT
      TCGATCAAAG TCTCAATGGT ACATGCATAA GGTGATCGAC CCAAGACATA
    AAGTCAAGAA AGCTATGCCG AATCCCATTA

721 GTCAGTACAG GAAAAAACT GTGCAAGTGA GCACCTGATT CCGTTGCCTT
    GGCTTAACTC TAAAGCTCCA TGTCTGGGC
      CAGTCATGTC CTTTTTTTGA CACGTTCACT CGTGGACTAA GGCAACGGAA
    CCGAATTGAG ATTTGAGGT ACAGGACCCG

801 CTAAAATCGT ATAAAATCTG GA
    GATTTTAGCA TATTTTAGAC CT

```


FIG. 18.

```

      +3
N L L T E E V R L Y
M N I F L L
]-----

1 AGGAAATCAA ATTAGGATAA GATTTGTATC TGATGAATAT TTTCCTTCTG
AACCTTCTAA CAGAGGAGGT AAGATTATAC
TCCTTTAGTT TAATCCTATT CTAAACATAG ACTACTTATA AAAGGAAGAC
TTGGAAGATT GTCTCCTCCA TTCTAATATG

      +3 S C T P R N F S V S I R E E L K R
T D T I F W P G C L
]-----

81 AGCTGCACAC CTCGTAACCT CTCAGTGTCC ATAAGGGAAG AACTAAAGAG
AACCGATACC ATTTTCTGGC CAGGTTGTCT
TCGACGTGTG GAGCATTGAA GAGTCACAGG TATTCCCTTC TTGATTCTC
TTGGCTATGG TAAAAGACCG GTCCAACAGA
-2
<-----

      +3 L V K R C G G N C A C C L H N C N
E C Q C V P S K V
]-----

161 CCTGGTTAAA CGCTGTGGTG GGAAGTGTGC CTGTTGTCTC CACAATTGCA
ATGAATGTCA ATGTGTCCCA AGCAAAGTTA
GGACCAATTT GCGACACCAC CCTTGACACG GACAACAGAG GTGTTAACGT
TACTTACAGT TACACAGGGT TCGTTTCAAT
-2
]-----

      +3 T K K Y H E V L Q L R P K T G V R
G L H K S L T D V A
]-----

      +1
D C T N H S P T W P
V S G
]-----

241 CTAAAAAATA CCACGAGGTC CTTCAGTTGA GACCAAAGAC CGGTGTCAGG
GGATTGCACA AATCACTCAC CGACGTGGCC
GATTTTTTAT GGTGCTCCAG GAAGTCAACT CTGGTTTCTG GCCACAGTCC
CCTAACGTGT TTAGTGAGTG GCTGCACCGG

```

FIG. 18 (CONTINUED 1).

```

-2 -----
-----[
+3 L E H H E E C D C V C R G S T G G
-----
>
+2 V Q R E H R R
I A A S P P A A L A ]-----
-----
+1 W S T M R S V T V C A E G A Q E D
S R I T T S S S C
-----
321 CTGGAGCACC ATGAGGAGTG TGA CTGTGTG TGCAGAGGGA GCACAGGAGG
ATAGCCGCAT CACCACCAGC AGCTCTTGCC
GACCTCGTGG TACTCCTCAC ACTGACACAC ACGTCTCCCT CGTGTCTCTC
TATCGGCGTA GTGGTGGTCG TCGAGAACGG
-----
+2 Q S C A V Q W L I L L E N V C V I
S I L N L S C L L Q
-----
+1 P E L C S A V A D S I R E R M R Y
L H P
-----
----->
401 CAGAGCTGTG CAGTGCAGTG GCTGATTCTA TTAGAGAACG TATGCGTTAT
CTCCATCCTT AATCTCAGTT GTTGTCTTCA
GTCTCGACAC GTCACGTCAC CGACTAAGAT AATCTCTTGC ATACGCAATA
GAGGTAGGAA TTAGAGTCAA CAAACGAAGT
-----
+2 G P F I F R I Y S A F
----->
481 AGGACCTTTC ATCTTCAGGA TTTACAGTGC ATTCTGAAAG AGGAGACATC
AAACAGAATT AGGAGTTGTG CAACAGCTCT
TCCTGGAAAG TAGAAGTCCT AAATGTCACG TAAGACTTTC TCCTCTGTAG
TTTGTCTTAA TCCTCAACAC GTTGTGCGAGA
-----
561 TTTGAGAGGA GGCCTAAAGG ACAGGAGAAA AGGTCTTCAA TCGTGGAAG
AAAATTAAAT GTTGTATTAA ATAGATCACC
AAACTCTCCT CCGGATTTC TGTCCTCTTT TCCAGAAGTT AGCACCTTTC
TTTTAATTAA CAACATAATT TATCTAGTGG
-----
641 AGCTAGTTTC AGAGTTACCA TGTACGTATT CCACTAGCTG GGTCTGTAT
TTCAGTTCTT TCGATACGGC TTAGGGTAAT
TCGATCAAAG TCTCAATGGT ACATGCATAA GGTGATCGAC CCAAGACATA
AAGTCAAGAA AGCTATGCCG AATCCCATTA
-----
721 GTCAGTACAG GAAAAAACT GTGCAAGTGA GCACCTGATT CCGTTGCCTT
GGCTTAACTC TAAAGCTCCA TGTCCTGGGC

```

FIG. 18 (CONTINUED 2).

CAGTCATGTC CTTTTTTTGA CACGTTCACT CGTGGAATAA GGCAACGGAA
CCGAATTGAG ATTTTCGAGGT ACAGGACCCG

801 CTAAATCGT ATAAATCTG GATTTTTTTN TTTTTTTTG CGCATATTCA
CATATGTAAA CCAGAACATT CTATGTACTA
GATTTTAGCA TATTTTAGAC CTAATAAAN AAAAAAAC GCGTATAAGT
GTATACATTT GGTCTTGTA GATACATGAT

881 CAAACCTGGT TTTTAAAAAG GAACTATGTT GCTATGAATT AAACCTGTGT
CGTGCTGATA GGACAGACTG GATTTTTCAT
GTTTGGACCA AAAATTTTTC CTTGATACAA CGATACTTAA TTTGAACACA
GCACGACTAT CCTGTCTGAC CTAATAAGTA
-3 <-----

961 ATTTCTTATT AAAATTTCTG CCATTTAGAA GAAGAGAACT ACATTCATGG
TTTGGAAGAG ATAAACCTGA AAAGAAGAGT
TAAAGAATAA TTTTAAAGAC GGTAAATCTT CTTCTCTTGA TGTAAGTACC
AAACCTTCTC TATTTGGACT TTTCTTCTCA
-3 -----

1041 GGCCTTATCT TCACTTTATC GATAAGTCAG TTTATTTGTT TCATTGTGTA
CATTTTTATA TTCTCCTTTT GACATTATAA
CCGGAATAGA AGTGAAATAG CTATTCAGTC AAATAAACAA AGTAACACAT
GTAAAAATAT AAGAGGAAAA CTGTAATATT
-3 ----- [

1121 CTGTTGGCTT TTCTAATCTT GTTAAATATA TCTATTTTTA CCAAAGGTAT
TTAATATTCT TTTTATGAC AACTTAGATC
GACAACCGAA AAGATTAGAA CAATTTATAT AGATAAAAAAT GGTTCCTATA
AATTATAAGA AAAAATACTG TTGAATCTAG

1201 AACTATTTTT AGCTTGGTAA ATTTTCTAA ACACAATTGT TATAGCCAGA
GGAACAAAGA TGATATAAAA TATTGTTGCT
TTGATAAAAA TCGAACCATT TAAAAAGATT TGTGTTAACA ATATCGGTCT
CCTTGTTTCT ACTATATTTT ATAACAACGA

1281 CTGACAAAAA TACATGTATT TCATTCTCGT ATGGTGCTAG AGTTAGATTA
ATCTGCATTT TAAAAAATG AATTGGAATA
GACTGTTTTT ATGTACATAA AGTAAGAGCA TACCACGATC TCAATCTAAT
TAGACGTAAA ATTTTTTGAC TTAACCTTAT

1361 GAATTGGTAA GTTGCAAAGA CTTTTTGAAA ATAATTAAAT TATCATATCT
TCCATTCTTG TTATTGGAGA TGAAAAATAA
CTTAACCATT CAACGTTTCT GAAAACTTT TATTAATTTA ATAGTATAGA
AGGTAAGGAC AATAACCTCT ACTTTTATT

1441 AAGCAACTTA TGAAAGTAGA CATTGAGATC CAGCCATTAC TAACCTATTC
CTTTTTTGGG GAAATCTGAG CCTAGCTCAG
TTCGTTGAAT ACTTTCATCT GTAAGTCTAG GTCGGTAATG ATTGGATAAG
GAAAAAACCC CTTTAGACTC GGATCGAGTC

FIG. 18 (CONTINUED 3).

1521 AAAAACATAA AGCACCTTGA AAAAGACTTG GCAGCTTCCT GATAAAGCGT
GCTGTGCTGT GCAGTAGGAA CACATCCTAT
TTTTTGTTT TCGTGGAAC TTTTCTGAAC CGTCGAAGGA CTATTTGCA
CGACACGACA CGTCATCCTT GTGTAGGATA

1601 TTATTGTGAT GTTGTGGTTT TATTATCTTA AACTCTGTTC CATACACTTG
TATAAATACA TGGATATTTT TATGTACAGA
AATAACACTA CAACACCAAA ATAATAGAAT TTGAGACAAG GTATGTGAAC
ATATTTATGT ACCTATAAAA ATACATGTCT

1681 AGTATGTCTC TTAACCAGTT CACTTATTGT ACCTGG
TCATACAGAG AATTGGTCAA GTGAATAACA TGGACC

FIG. 19. DNA and polypeptide sequence used for mammalian cell expression

```

+1      m s l f g l l l l t s a l a g q r
1  GGATCCAAAA TGACCTCTT CGGGCTTCTC CTGCTGACAT CTGCCCTGGC CGGCCAGAGA

+1  q g t q a e s n l s s k f o f s s n k e
61  CAGGGGACTC AGGCGGAATC CAACCTGAGT AGTAAATTC AGTTTCCAG CAACAAGGAA

+1  Q N G V Q D P Q H E R I I T V S T N G S
121 CAGAACGGAG TACAAGATCC TCAGCATGAG AGAATTATTA CTGTGTCTAC TAATGGAAGT

+1  I H S P R F P H T Y P R N T V L V W R L
181 ATTCACAGCC CAAGGTTTCC TCATACTTAT CCAAGAAATA CGGTCTTGGT ATGGAGATTA

+1  V A V E E N V W I Q L T F D E R F G L E
241 GTAGCAGTAG AGGAAAATGT ATGGATACAA CTTACGTTTG ATGAAAGATT TGGGCTTGAA

+1  D P E D D I C K Y D F V E V E E P S D G
301 GACCCAGAAG ATGACATATG CAAGTATGAT TTTGTAGAAG TTGAGGAACC CAGTGATGGA

+1  T I L G R W C G S G T V P G K Q I S K G
361 ACTATATTAG GGCCTGGTG TGGTCTGGT ACTGTACCAG GAAAACAGAT TTCTAAAGGA

+1  N Q I R I R F V S D E Y F P S E P G F C
421 AATCAAATTA GGATAAGATT TGTATCTGAT GAATATTTTC CTTCTGAACC AGGGTTCTGC

+1  I H Y N I V M P Q F T E A V S P S V L P
481 ATCCACTACA ACAATGTCAT GCCACAATC ACAGAAGCTG TGAGTCCTTC AGTGCTACCC

+1  P S A L P L D L L N N A I T A P S T L E
541 CCTTCAGCTT TGCCACTGGA CTGCTTAAT AATGCTATAA CTGCCTTTAG TACCTTGAA

+1  D L I R Y L E P E R W Q L D L E D L Y R
601 GACCTTATTC GATATCTTGA ACCAGAGAGA TGGCASTGG ACTTAGAAGA TCTATATAGG

+1  P T W Q L L G K A F V F G R K S R V V D
661 CCAACTGGC AACTTCTTGG CAAGGCTTTT GTTTTGGAA GAAAATCCAG AGTGGTGGAT

+1  L N L L T E E V R L Y S C T P R N F S V
721 CTGAACCTTC TAACAGAGGA GGTAAGATTA TACAGCTGCA CACCTCGTAA CTTCTCAGTG

+1  S I R E E L K R T D T I F W P G C L L V
781 TCCATAAGGG AAGAACTAAA GAGAACCGAT ACCATTTTCT GGCCAGGTG TCTCCTGGTT

+1  K R C G G N C A C C L H N C N E C Q C V
841 AAACGCTGTG GTGGAACTG TGCCTGTTGT CTCCACAATT GCAATGAATG TCAATGTGTC

+1  P S K V T K K Y H E V L Q L R P K T G V
901 CCAAGCAAAG TTAATAAAAA ATACCACGAG GTCCTTCAGT TGAGACCAA GACCGGTGTC

+1  R G L H K S L T D V A L E H H E E C D C
961 AGGGGATTGC ACAAATCACT CACCGACGTG GCCCTGGAGC ACCATGAGGA GTGTGACTGT

+1  V C R G S T G G S R G P F E G K P I P N
1021 GTGTGCAGAG GGAGCACAGG AGGATCTAGA GGGCCCTTGG AAGGTAAGCC TATCCCTAAC

+1  P L L G L D S T R T G H H H H H H
1081 CCTCTCCTCG GTCTCGATTC TACGCGTACC GTTCATCATC ACCATCACCA TTGA

```

FIG. 20. DNA and polypeptide sequence used for baculovirus/insect cell expression

1 GAATTCAAAG GCGTGTATTT TACTGTTTTC GTAACAGTTT TGTAAATAAA AAACCTATAA
 +3 m k f l v n v a l v f m v v y i s y i
 61 ATATGAAATT CTTAGTCAAC GTTGCCCTTG TTTTATGGT CGTATACATT TCTTACATCT
 +3 y a D P E S H H H H H E S N L S S K F
 121 ATGCGGATCC GGAGTCTCAC CATCACCACC ATCATGAATC CAACCTGAGT AGTAAATTCC
 +3 Q F S S N K E Q N G V Q D P Q H E R I I
 181 AGTTTTCCAG CAACAAGGAA CAGAACGGAG TACAAGATCC TCAGCATGAG AGAATTATTA
 +3 T V S T N G S I H S P R F P H T Y P R N
 241 CTGTGTCTAC TAATGGAAGT ATTCACAGCC CAAGGTTTCC TCATACTTAT CCAAGAAATA
 +3 T V L V W R L V A V E E N V W I Q L T F
 301 CGGTCTTGGT ATGGAGATTA GTAGCAGTAG AGGAAAATGT ATGGATACAA CTTACGTTTG
 +3 D E R F G L E D P E D D I C K Y D F V E
 361 ATGAAAGATT TGGGCTTGAA GACCCAGAAG ATGACATATG CAAGTATGAT TTTGTAGAAG
 +3 V E E P S D G T I L G R W C G S G T V P
 421 TTGAGGAACC CAGTGATGGA ACTATATTAG GCGCTGGTG TGGTCTGGT ACTGTACCAG
 +3 G K Q I S K G N Q I R I R F V S D E Y F
 481 GAAAACAGAT TTCTAAAGGA AATCAAATTA GGATAAGATT TGTATCTGAT GAATATTTTC
 +3 P S E P G F C I H Y N I V M P Q F T E A
 541 CTTCTGAACC AGGTTCTGTC ATCCACTACA ACATTGTCAT GCCACAATTC ACAGAAGCTG
 +3 V S P S V L P P S A L P L D L L N N A I
 601 TGAGTCCTTC AGTGCTACCC CTTTCAGCTT TGCCACTGGA CCTGCTTAAT AATGCTATAA
 +3 T A F S T L E D L I R Y L E P E R W Q L
 661 CTGCCCTTAG TACCTTGGA GACCTTATTC GATATCTTGA ACCAGAGAGA TGGCAGTTGG
 +3 D L E D L Y R P T W Q L L G K A F V F G
 721 ACTTAGAAGA TCTATATAGG CCAACTTGGC AACTTCTTGG CAAGGCTTTT GTTTTTGGAA
 +3 R K S R V V D L N L L T E E V R L Y S C
 781 GAAAATCCAG AGTGGTGGAT CTGAACCTTC TAACAGAGGA GGTAAGATTA TACAGCTGCA
 +3 T P R N F S V S I R E E L K R T D T I F
 841 CACCTCGTAA CTTCTCAGTG TCCATAAGGG AAGAACTAAA GAGAACCGAT ACCATTTTCT
 +3 W P G C L L V K R C G G N C A C C L H N
 901 GGCCAGGTTG TCTCTGGT AAACGCTGTG GTGGGAAC TGCCCTGTTGT CTCCACAATT
 +3 C N E C Q C V P S K V T K K Y H E V L Q
 961 GCAATGAATG TCAATGTGTC CCAAGCAAAG TTACTAAAAA ATACCACGAG GTCCTTCAGT
 +3 L R P K T G V R G L H K S L T D V A L E
 1021 TGAGACCAAAA GACC3GTGTC AGGGGATTGC ACAAATCACT CACCGACGTG GCCCTGGAGC
 +3 H H E E S D C V C R G S T G G
 1081 ACCAT3AGGA GGTGACTGT GTGTGCAGAG GGAGCACAGG AGGATAGCTC TAGA

FIG. 21. DNA and polypeptide sequence used for *E. coli* expression

+3 Q T N S S S N N N N N N N N N L G I
 1 CGCAGACTAA TTCGAGCTCG AACAAACAACA ACAATAACAA TAACAACAAC CTCGGGATCG
 +3 E G R I S E F E S N L S S K F Q F S S N
 61 AGGGAAGGAT TTCAGAATTC GAATCCAACC TGAGTAGTAA ATTCCAGTTT TCCAGCAACA
 +3 K E Q N G V Q D P Q H E R I I T V S T N
 121 AGGAACAGAA CGGAGTACAA GATCCTCAGC ATGAGAGAAT TATTACTGTG TCTACTAATG
 +3 G S I H S P R F P H T Y P R N T V L V W
 181 GAAGTATTCA CAGCCCAAGG TTTCCTCATA CTTATCCAAG AAATACGGTC TTGGTATGGA
 +3 R L V A V E E N V W I Q L T F D E R F G
 241 GATTAGTAGC AGTAGAGGAA AATGTATGGA TACAACCTAC GTTTGATGAA AGATTGCGG
 +3 L E D P E D D I C K Y D F V E V E E P S
 301 TTGAAGACCC AGAAGATGAC ATATGCAAGT ATGATTTTGT AGAAGTTGAG GAACCCAGTG
 +3 D G T I L G R W C G S G T V P G K Q I S
 361 ATGGAAGTAT ATTAGGGCGC TGGTGTGGTT CTGGTACTGT ACCAGGAAAA CAGATTTCTA
 +3 K G N Q I R I R F V S D E Y F P S E P G
 421 AAGGAAATCA AATTAGGATA AGATTTGTAT CTGATGAATA TTTTCCTTCT GAACCCAGGT
 +3 F C I H Y N I V M P Q F T E A V S P S V
 481 TCTGCATCCA CTACAACATT GTCATGCCAC AATTCACAGA AGCTGTGAGT CCTTCAGTGC
 +3 L P P S A L P L D L L N N A I T A F S T
 541 TACCCCTTC AGCTTTGCCA CTGGACCTGC TTAATAATGC TATAACTGCC TTTAGTACCT
 +3 L E D L I R Y L E P E R W Q L D L E D L
 601 TGGAAGACCT TATTCGATAT CTTGAACCAG AGAGATGGCA GTTGGACTTA GAAGATCTAT
 +3 Y R P T W Q L L G K A F V F G R K S R V
 661 ATAGGCCAAC TTGGCAACTT CTTGGCAAGG CTTTGTGTTT TGGAAGAAAA TCCAGAGTGG
 +3 V D L N L L T E E V R L Y S C T P R N F
 721 TGGATCTGAA CTTCTAACA GAGGAGGTAA GATTATACAG CTGCACACCT CGTAACCTCT
 +3 S V S I R E E L K R T D T I F W P G C L
 781 CAGTGTCAT AAGGGAAGAA CTAAAGAGAA CCGATACCAT TTTCTGGCCA GGTGTCTCC
 +3 L V X R C G G N C A C C L H N C N E C Q
 841 TGGTTAAACG CTGTGGTGGG AACTGTGCCT GTTGTCTCCA CAATTGCAAT GAATGTCAAT
 +3 C V F S K V T K K Y H E V L Q L R P K T
 901 GTGTCCCAAG CAAAGTTACT AAAAAATACC ACGAGGTCTT TCAGTTGAGA CCAAAGACCG
 +3 G V R G L H K S L T D V A L E H H E E C
 961 GTGTCAAGGG ATTGCACAAA TCACTACCG ACCTGGCCCT GGAGCACCAT GAGGAGTGTG
 +3 D C V C R G S T G G H H H H H H
 1021 ACTGTGTGTG CAGAGGGAGC ACAGGAGGAC ATCATCACCA TCACCATTGA TCTAGAGTCG
 1081 ACCTGCAGGC AAGCTT

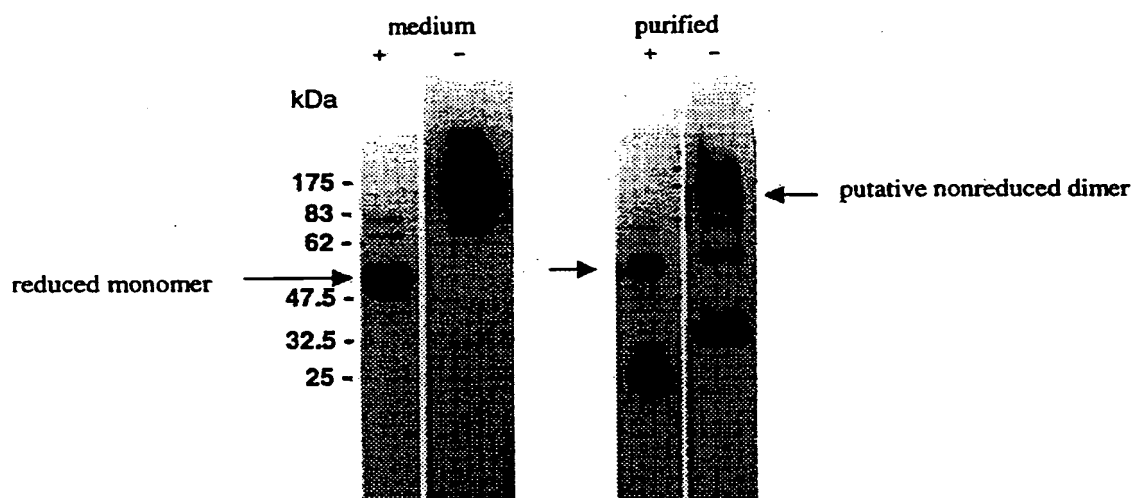
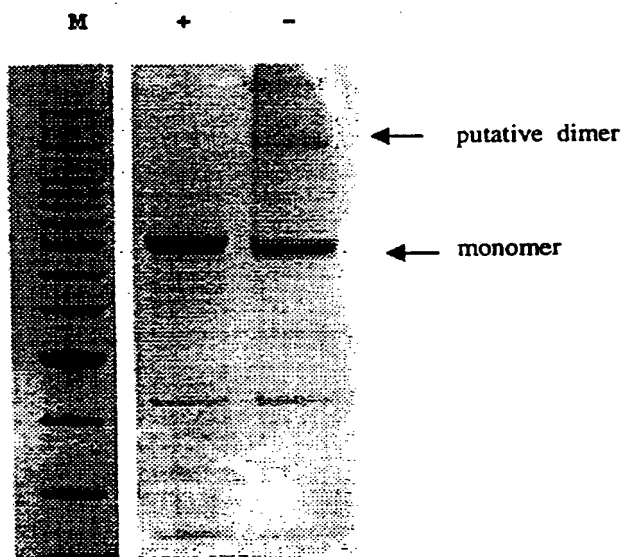
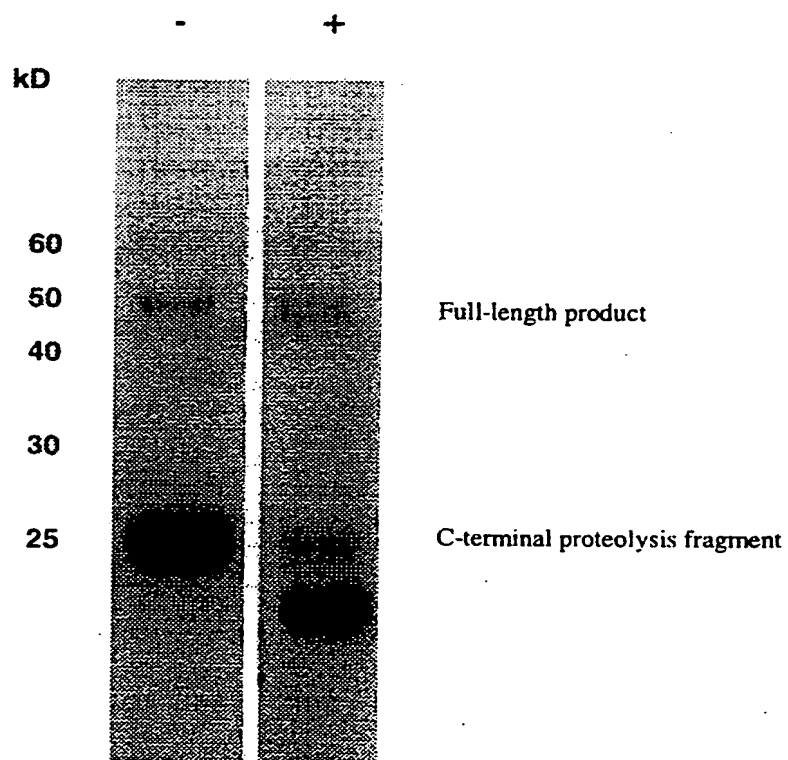
FIG. 22. Disulphide-linked dimerisation of VEGF-X**(A) Mammalian cell expression****(B) *E.coli* expression**

FIG. 23. Glycosylation of VEGF-X



*FIG. 24.*DNA and polypeptide sequence used for *E.coli* expression of the PDGF-like domain

```
+3           M R G S H H H H H H G M A S M
1  AAGGAGATAT ACATATGCGG GGTTCATC ATCATCATCA TCATGGTATG GCTAGCATGA

+3 T G G O O M G R D L Y D D D D K D P G R
61 CTGGTGGACA GCAAATGGGT CGGGATCTGT ACGACGATGA CGATAAGGAT CCGGGAAGAA

+3 K S R V V D L N L L T E E V R L Y S C T
121 AATCCAGAGT GGTGGATCTG AACCTTCTAA CAGAGGAGGT AAGATTATAC AGCTGCACAC

+3 P R N F S V S I R E E L K R T D T I F W
181 CTCGTAACCT CTCAGTGTC ATAAGGGAAG AACTAAAGAG AACCGATACC ATTTTCTGGC

+3 P G C L L V K R C G G N C A C C L H N C
241 CAGGTTGTCT CTTGGTTAAA CGCTGTGGTG GGAAGTGTGC CTGTTGTCTC CACAATTGCA

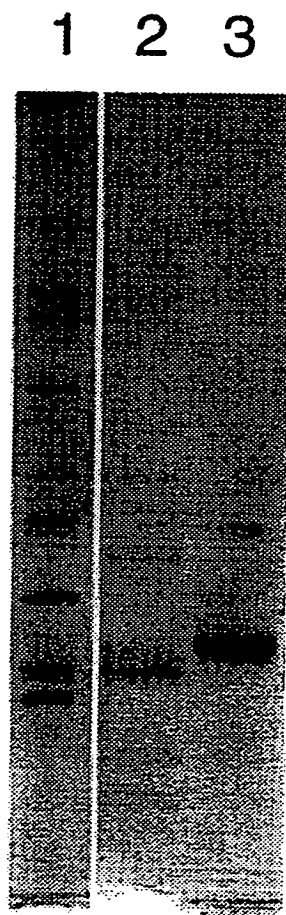
+3 N E C Q C V P S K V T K K Y H E V L Q L
301 ATGAATGTCA ATGTGTCCCA AGCAAAGTTA CTAAAAAATA CCACGAGGTC CTTCAATTGA

+3 R P K T G V R G L H K S L T D V A L E H
361 GACCAAAGAC CGGTGTCAGG GGATTGCACA AATCACTCAC CGACGTGGCC CTGGAGCACC

+3 H E E C D C V C R G S T G G
421 ATGAGGAGTG TGAATGTGTG TGCAGAGGGA GCACAGGAGG ATAATGAATT CGAAGCTTGA

481 TCCGGCTGCT AACAAAGCCC
```

FIG. 25. Expression of PDGF domain in *E.coli*



*FIG. 26.*DNA and polypeptide sequence used for *E.coli* expression of the CUB-like domain

```
+2      M A M D I G I N S D P E S H H H H H H
1  GGCGATGGCC ATGGATATCG GAATTAATTC GGATCCGGAG TCTCACCATC ACCACCATCA

+2      E S N L S S K F Q F S S N K E Q N G V Q
61 TGAATCCAAC CTGAGTAGTA AATTCCAGTT TTCCAGCAAC AAGGAACAGA ACGGAGTACA

+2      D P Q H E R I I T V S T N G S I H S P R
121 AGATCCTCAG CATGAGAGAA TTATTACTGT GTCTACTAAT GGAAGTATTC ACAGCCCAAG

+2      F P H T Y P R N T V L V W R L V A V E E
181 GTTTCCTCAT ACTTATCCAA GAAATACGGT CTTGGTATGG AGATTAGTAG CAGTAGAGGA

+2      N V W I Q L T F D E R F G L E D P E D D
241 AAATGTATGG ATACAACTTA CGTTTGATGA AAGATTGGG CTTGAAGACC CAGAAGATGA

+2      I C K Y D F V E V E E P S D G T I L G R
301 CATATGCAAG TATGATTTTG TAGAAGTTGA GGAACCCAGT GATGGAAC TAATTAGGGCG

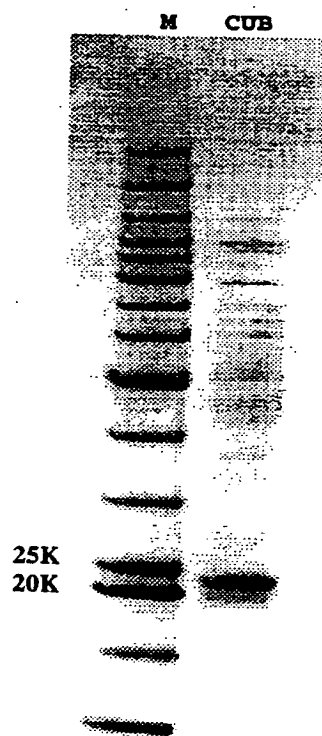
+2      W C G S G T V P G K Q I S K G N Q I R I
361 CTGGTGTGGT TCTGGTACTG TACCAGGAAA ACAGATTCTT AAAGGAAATC AAATTAGGAT

+2      R F V S D E Y F P S E P G F C I H Y N I
421 AAGATTGTGA TCTGATGAAT ATTTTCCTTC TGAACCAGGG TTCTGCATCC ACTACAACAT

+2      V M P C F T E A V
481 TGTGATGCCA CAATTCACAG AAGCTGTGTA GTCGAGCTCC GTCGACAAGC TTGCGGCCGC

541 ACTCGAGCAC
```

FIG. 27. Expression of the CUB domain in *E.coli*



*FIG. 28. The Effect of Truncated VEGF-X
(CUB domain) on HUVEC Proliferation.*

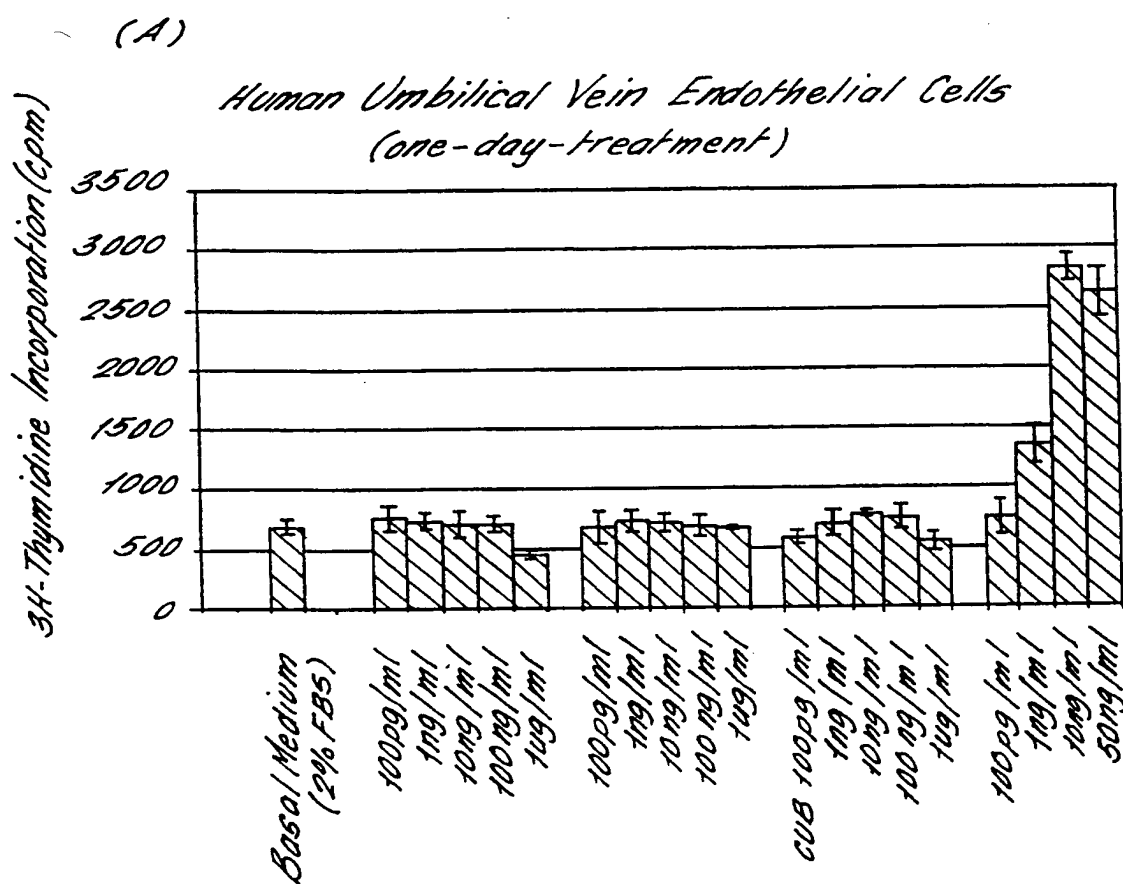


FIG. 28 (CONTINUED 1).

(B)

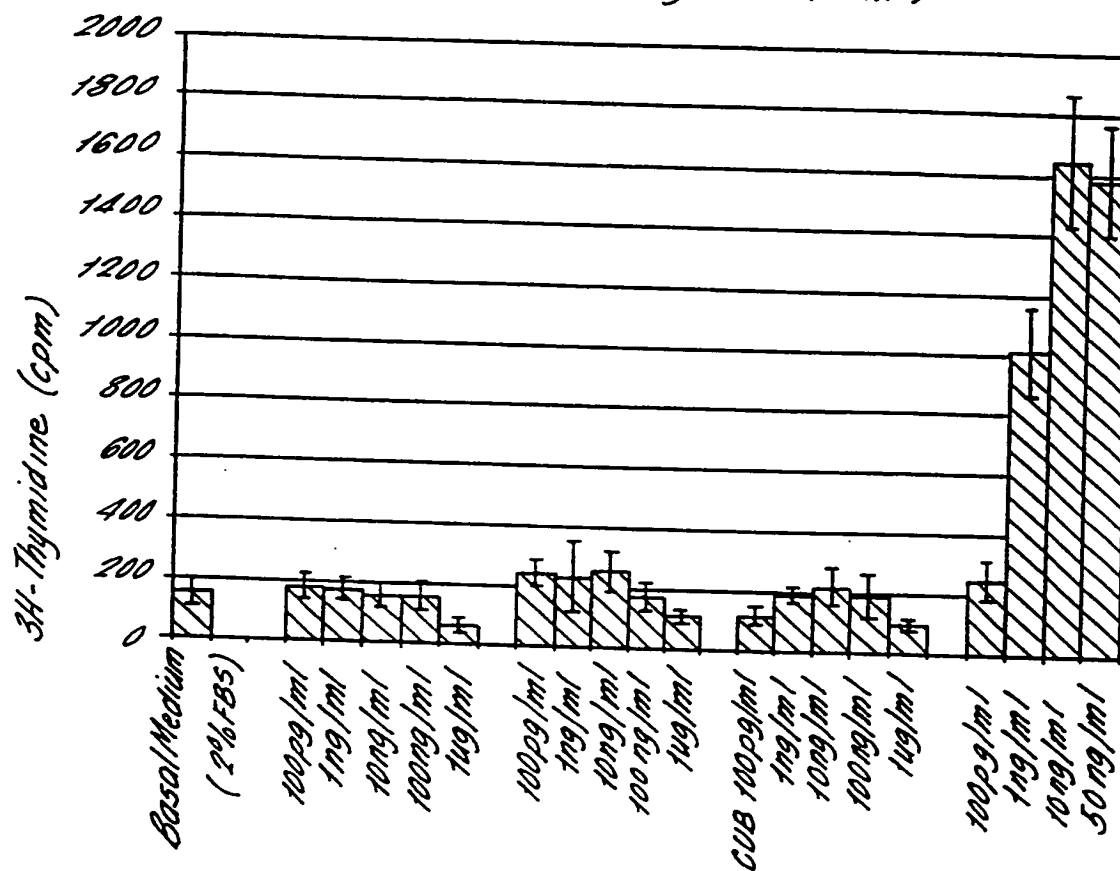
Human Umbilical Vein Endothelial Cells (24-hour-starving Followed by one-day-treatment)

FIG. 28 (CONTINUED 2).

(C)

The effect of VEGF-A₁₆₅ and VEGF-X CUB domain on the proliferation of HUVEC (two-day-treatment).

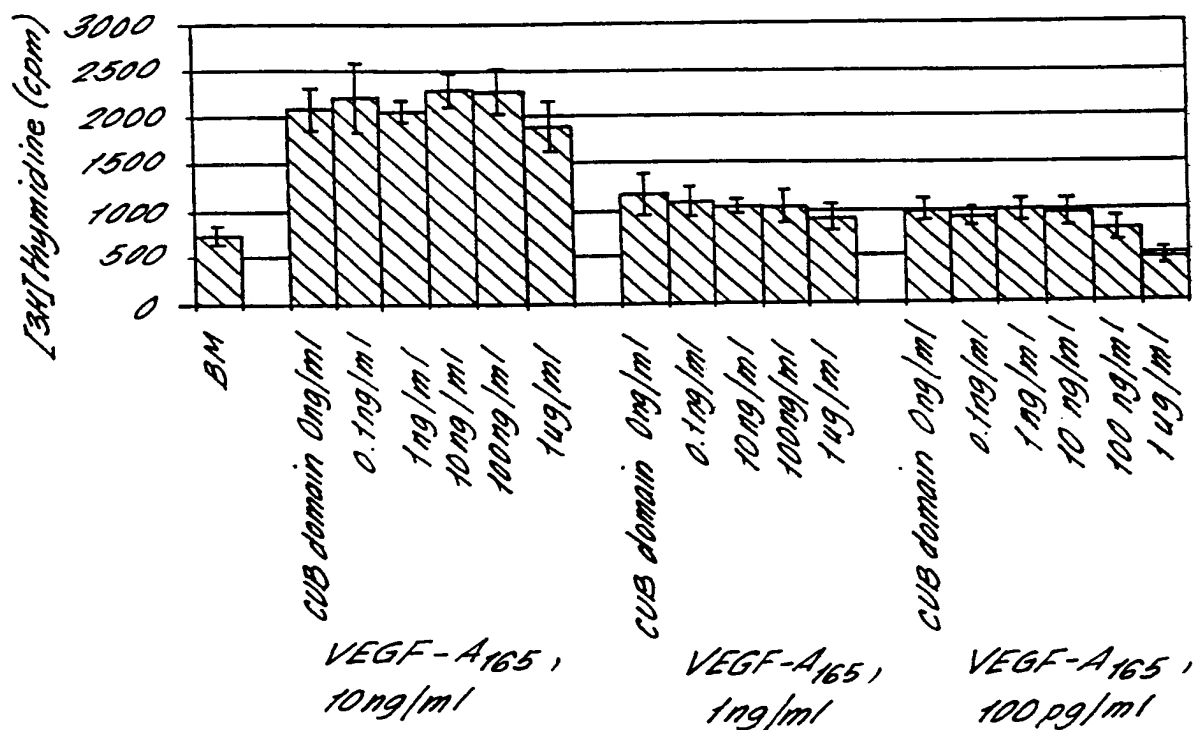


FIG. 29.
Tissue distribution of mRNA
(A) - Normal tissues

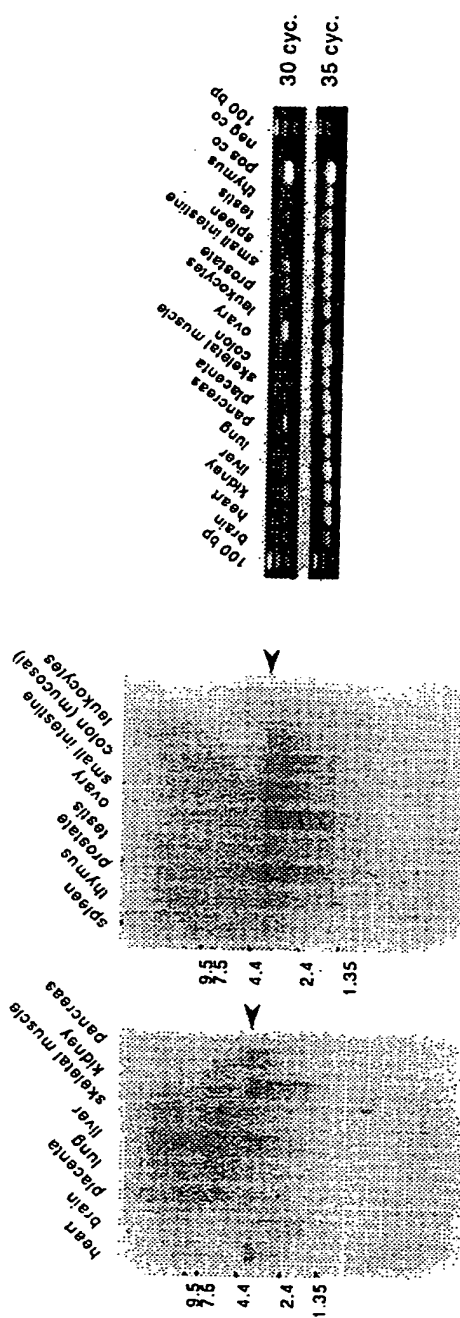
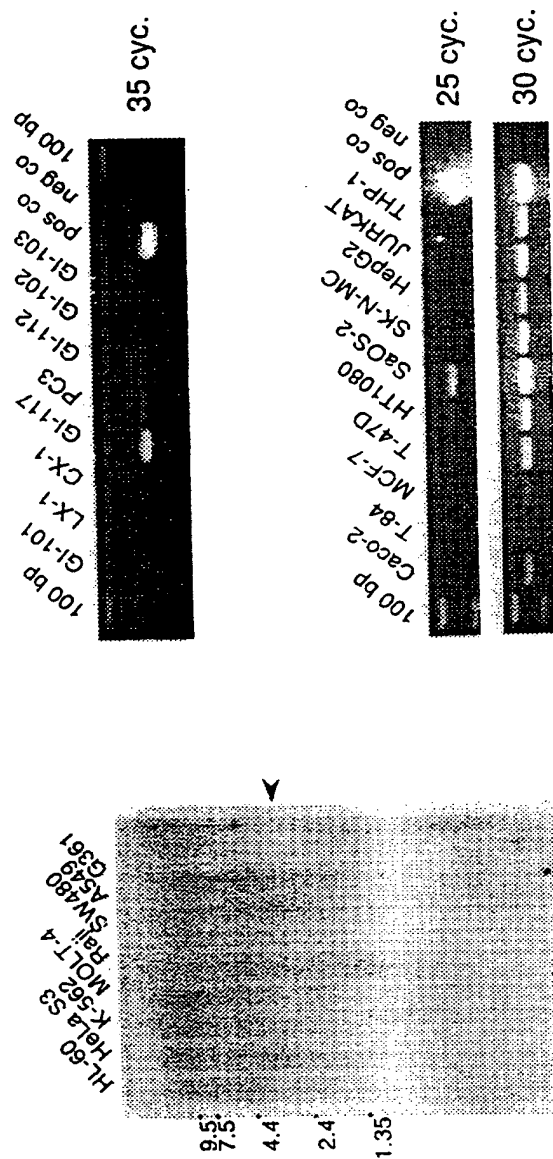


FIG. 29 (CONTINUED). (B)- Tumour tissue and cell lines



(A) - Genomic DNA sequences of 2 exons determined by sequencing

[illegible]

FIG. 30 (CONTINUED 1).

(B) - Location of splice sites within the cDNA sequence

1 GAATTCGCCC TTTGTTTAA ACCTTGGGAA CTGGTTCAGG TCCAGGTTTT GCTTTGATCC
 61 TTTTCAAAAA CTGGAGACAC AGAAGAGGGC TCTAGGAAAA AGTTTTGGAT GGGATTATGT
 121 GGAAACTACC CTGCGATTCT CTGCTGCCAG AGCAGGCTCG GCGCTTCAC CCCAGTGCAG
 181 CCTTCCCCTG GCGGTGGTGA AAGAGACTCG GGAGTGGCTG CTTCCAAAGT GCCCGCCGTG
 +3 M S L F G L L L L T S
 241 AGTGAGCTCT CACCCAGTC AGCCAAATGA GCCTCTTCGG GCTTCTCCTG CTGACATCTG
 +3 A L A G Q R Q G T Q A E S N L S S K F Q
 301 CCCTGGCCGG CCGAGACAG GGGACTCAGG CGGAATCCAA CCTGAGTAGT AAATCCAGT
 +3 F S S N K E Q N G V Q D P Q H E R I I T
 361 TTTCCAGCAA CAAGGAACAG AACGGAGTAC AAGATCCTCA GCATGAGAGA ATTATTACTG
 +3 V S T N G S I H S P R F P H T Y P R N T
 421 TGTCTACTAA TGGAAGTATT CACAGCCCAA GGTTCCTCA TACTTATCCA AGAAATACGG
 +3 V L V W R L V A V E E N V W I Q L T F D
 481 TCTTGGTATG GAGATTAGTA GCAGTAGAGG AAAATGTATG GATACACTT ACGTTTGATG
 +3 E R F G L E D P E D D I C K Y D F V E V
 541 AAAGATTGG GCTTGAAGAC CCAGAAGATG ACATATGCAA GTATGATTTT GTAGAAGTTG
 +3 E E P S D G T I L G R W C G S G T V P G
 601 AGGAACCCAG TGATGGAAT ATATTAGGGC GCTGGTGTGG TCTGGTACT GTACCAGGAA
 +3 K Q I S K G N Q I R I R F V S D E Y F P
 661 AACAGATTTC TAAAGGAAAT CAAATTAGGA TAAGATTGT ATCTGATGAA TATTTTCTT
 +3 S E P G F C I H Y N I V M P Q F T E A V
 721 CTGAACCAAG GTTCTGCATC CACTACAACA TTGTCATGCC ACAATTCACA GAAGCTGTGA
 +3 S P S V L P P S A L P L D L L N N A I T
 781 GTCCTTCAGT GCTACCCCT TCAGCTTTC CACTGGACCT GCTTAATAAT GCTATAACTG
 +3 A F S T L E D L I R Y L E P E R W Q L D
 841 CCTTTAGTAC CTGGAAGAC CTTATTGAT ATCTGAACC AGAGAGATGG CAGTTGGACT
 +3 L E D L Y R P T W Q L L G K A F V F G R
 901 TAGAAGATCT ATATAGGCCA ACTTGGAAC TTCTGGCAA GGCTTTTGT TTTGGAAGAA
 +3 K S R V V D L N L L T E E V R L Y S C T
 961 AATCCAGAGT GGTGGATCTG AACCTTCTAA CAGAGGAGT AAGATTATAC AGCTGCACAC
 +3 P R N F S V S I R E E L K R T D T I F W
 1021 CTCGTAACCT CTCAGTGTCC ATAAGGGAAG AACTAAGAG AACCGATACC ATTTCTGGC
 +3 P G C L L V K R C G G N C A C C L E N C
 1081 CAGGTGTCT CCTGGTTAAA CGCTGTGGTG GGAAGTGC CTGTTGTCTC CACAATIGCA
 +3 N E C Q C V P S K V T K K Y H E V L Q L
 1141 ATGAATGTCA ATGTGTCCCA AGCAAAGTGA CTAAAAATA CCACGAGTC CTTCAAGTGA

FIG. 30(CONTINUED 2).

+3 R P K T G V R G L H K S L T D V A L E H
1201 GACCAAAGAC CCGTGTGAGG GGATTGCACA AATCACTCAC CGACGTGGCC CTGGAGCACC

+3 H E E C D C V C R G S T G G
1261 ATGAGGAGTG TCACTGTGTG TGCAGAGGGA GCACAGGAGG ATAGCCGCAT CACCACCAGC

1321 AGCTCTTGCC CAGAGCTGTG CAGTGCAGTG GCTGATTCTA TTAGAGAACG TATGCGTTAT

1381 CTCCATCCTT AAICTCAGTT GTTTGCTTCA AGGACCTTTC ATCTTCAGGA TTTACAGTGC

1441 ATTCTGAAAG AEGAGACATC AAACAGAATT AGGAGTTGTG CAACAGCTCT TTTGAGAGGA

1501 GGCCTAAAGG ACAGGAGAAA AGGTCTTCAA TCGTGGAAAG AAAATTAAAT GTTGTATTAA

1561 ATAGATCACC AGCTAGTTTC AGAGTTACCA TGTACGTATT CCACTAGCTG GGTCTGTAT

1621 TTCAGTTCTT TCGATACGGC TTAGGGTAAT GTCAGTACAG GAAAAAACT GTGCAAGTGA

1681 GCACCTGATT CCGTTGCCTT GCTTAACTCT AAAGCTCCAT GTCCTGGGCC TAAAATCGTA

1741 TAAAATCTGG ATTTTTTTTT TTTTTTTTG CTCATATTCA CATATGTAAA CCAGAACATT

1801 CTATGTACTA CAAACCTGGT TTTTAAAAG GAACTATGTT GCTATGAATT AAACCTGTGT

1861 CATGCTGATA GGACAGACTG GATTTTTCAT ATTTCTTATT AAAATTCTG CCATTTAGAA

1921 GAAGAGAAGT ACATTCATGG TTTGGAAGAG ATAAACCTGA AAAGAAGAGT GGCCTTATCT

1981 TCACTTTATC GATAAGTCAG TTTATTTGTT TCATTGTGTA CATTTTATA TTCTCCTTTT

2041 GACATTATAA CTGTTGGCTT TTCTAATCTT GTTAAATATA TCTATTTTTA CCAAAGGTAT

2101 TTAATATTCT TTTTATGAC AACTTAGATC AACTATTTT AGCTTGGTAA ATTTTCTAA

2161 ACACAATTGT TATAGCCAGA GGAACAAAGA TGATATAAAA TATTGTTGCT CTGACAAAAA

2221 TACATGTATT TCATTCTCGT ATGGTGCTAG AGTTAGATTA ATCTGCATT TAAAAAAGT

2281 AATTGGAATA GAATGGTAA GTTGCAAAGA CTTTTTGAAA ATAATTAAAT TATCATATCT

2341 TCCATTCTG TTAATGGAGA TGAAATAAA AAGCAACTTA TGAAAGTAGA CATTGAGATC

2401 CAGCCATTAC TAACCTATTC CTTTTTGGG GAAATCTGAG CCTAGCTCAG AAAAACATAA

2461 AGCACCTTGA AAAAGACTTG GCAGCTTCCT GATAAAGCGT GCTGTGCTGT GCAGTAGGAA

2521 CACATCCTAT TTAATGTGAT GTTGTGGTTT TATTATCTTA AACTCTGTTT CATACTTG

2581 TATAAATACA TGGATATTTT TATGTACAGA AGTATGTCTC TTAACCAGTT CACTTATTGT

2641 ACCTGGAAGG GCGAATTCTG CAGATATC

FIG. 31.

*The Effect of FL-VEGF-X on HUVEC Proliferation:
(24-hour serum starvation followed by
one day-treatment)*

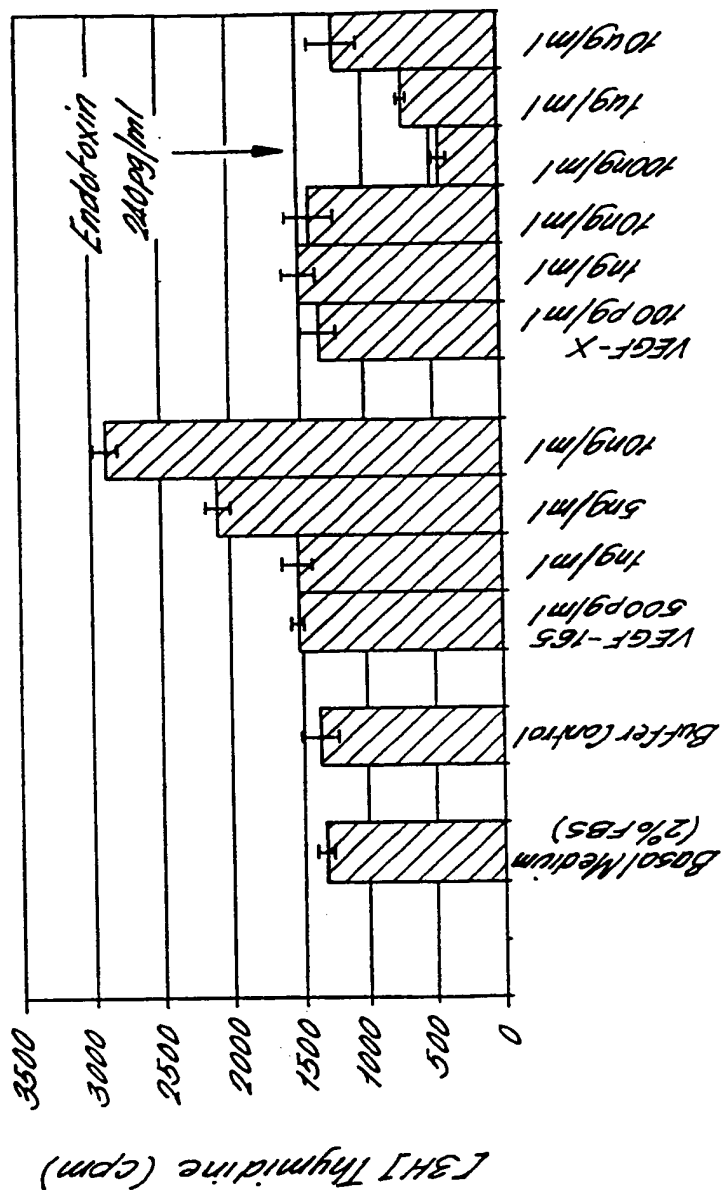


FIG. 32.
The Combined Effect of Truncated VEGF-X (CUB domain) and Human Recombinant VEGF₁₆₅ on HUVEC Proliferation: (24-hour serum starvation followed by two-day-treatment)

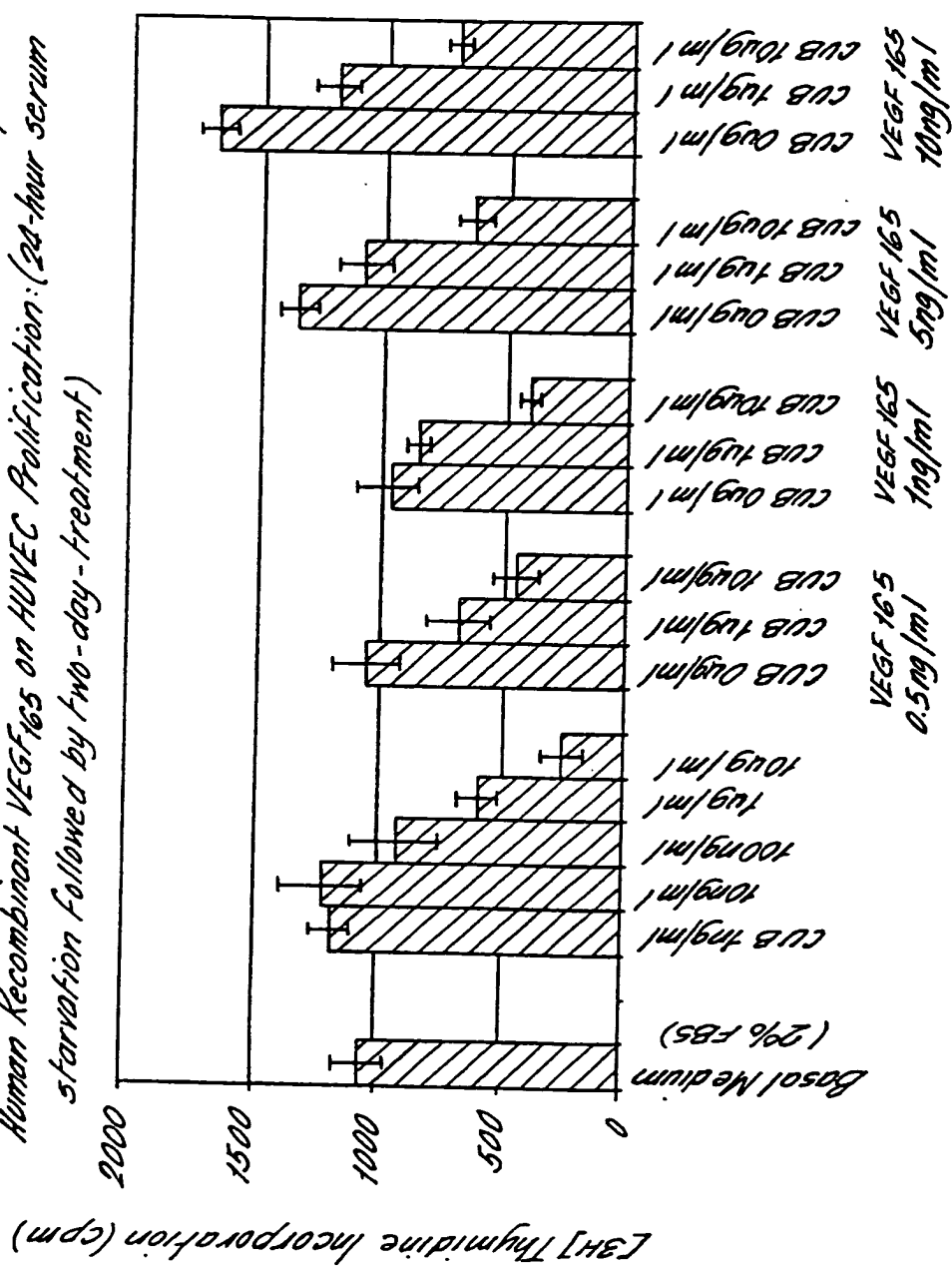


FIG. 33.
The Combined Effect of CUB Domain and Human Recombinant bFGF on HUVEC Proliferation: (24-hour serum starvation followed by two-day-treatment).

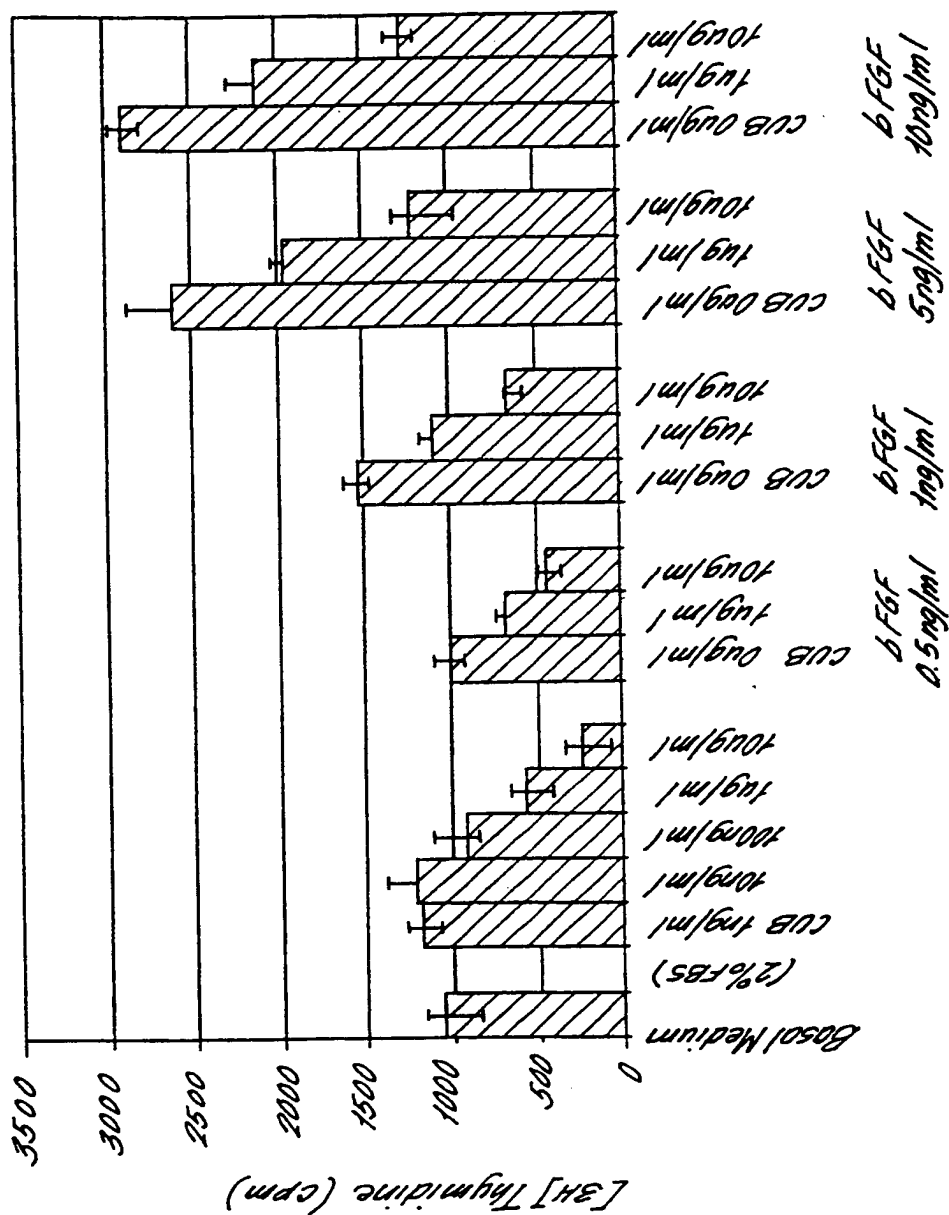


FIG. 34.
LDH Assay for Testing Cytotoxicity of CUB Domain or
CUB Domain with rhVEGF₁₆₅

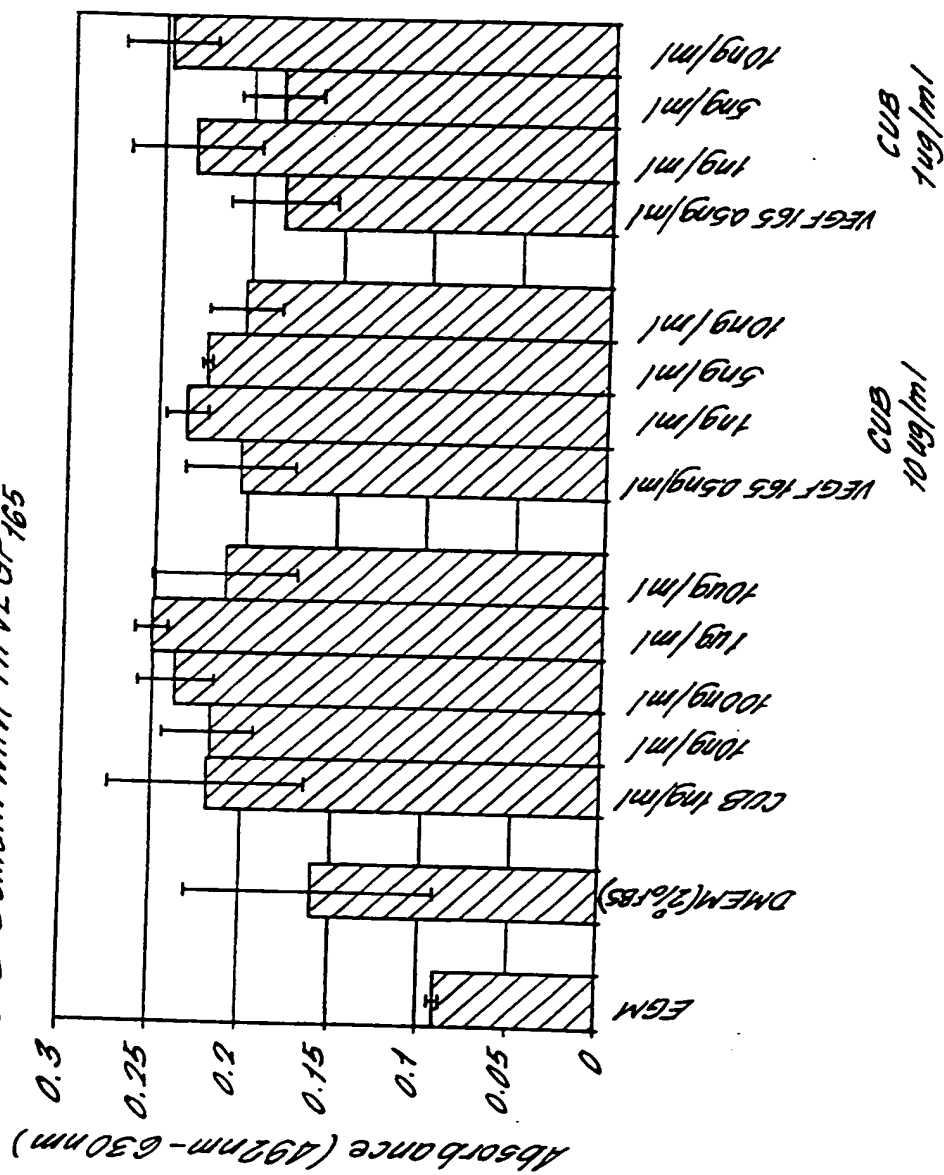
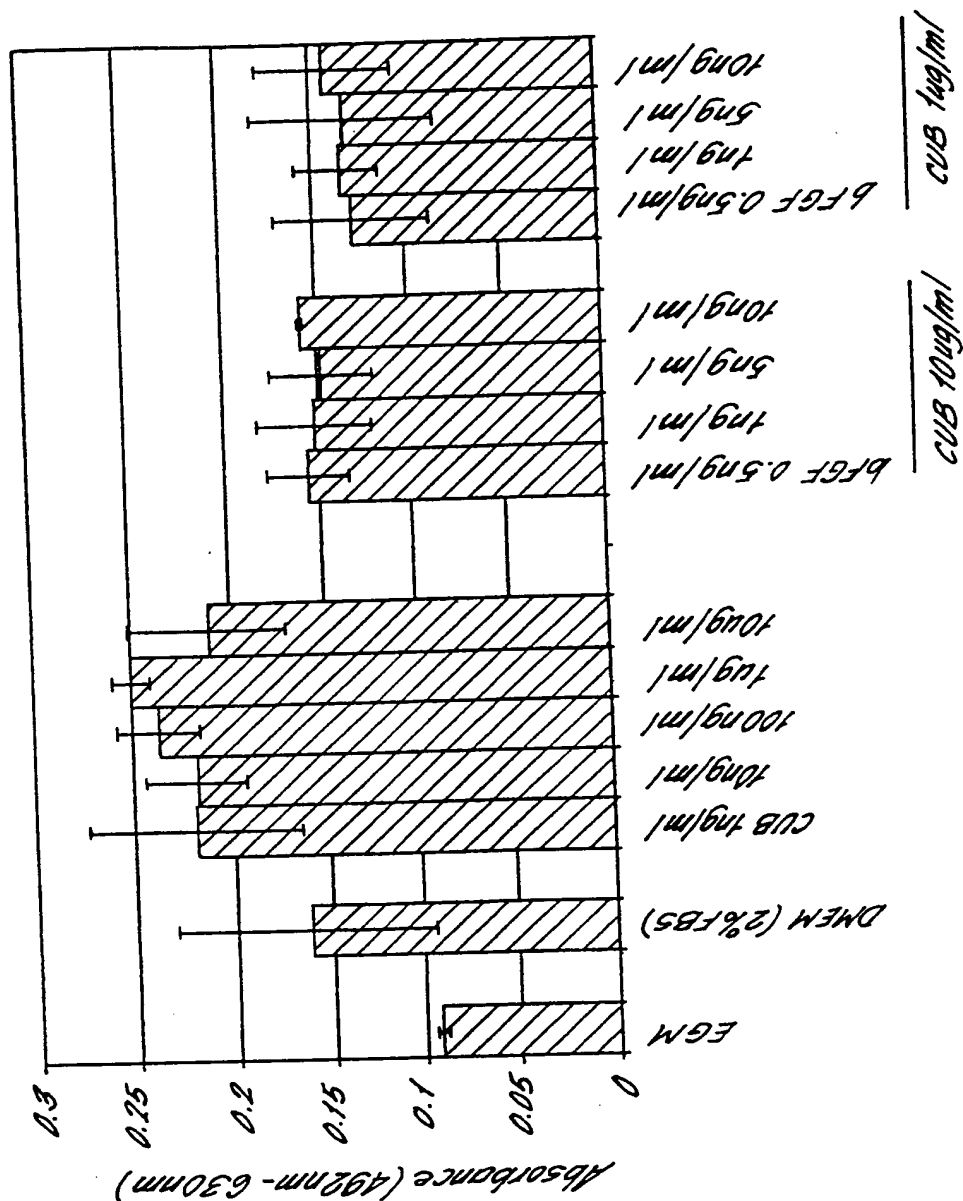


FIG. 35.
LDH Assay for Testing Cytotoxicity of CUB Domain or
CUB Domain with rh-bFGF



PCT/US99/30503

Applicant's or agent's file reference	B0192/7011W0	International application No. PCT/US99/30503
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INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>21</u> . line <u>15-16</u>	
B. IDENTIFICATION OF DEPOSIT	
Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution BELGIAN COORDINATED COLLECTIONS OF MICROORGANISMS (BCCM) TM LABORATORIUM VOOR MOLECULAIRE BIOLOGIE - PLASMIDENCOLLECTIE (LMBP)	
Address of depositary institution (including postal code and country) Universiteit Gent K.L. Ledeganckstraat 35 B-9000 Gent, Belgium	
Date of deposit 20 December 1999 (20.12.99)	Accession Number LMBP 3991
C. ADDITIONAL INDICATIONS (leave blank if not applicable)	
This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
For receiving Office use only	For International Bureau use only
<input type="checkbox"/> This sheet was received with the international application	<input checked="" type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	19 APRIL 2000 (19.04.00)
	Authorized officer Ellen Moyse

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LMBP-COLLECTION
Page 1 of Form BCCM™/LMBP/BP/4/99-23 Receipt in the case of an original deposit

Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure

Receipt in the case of an original deposit issued pursuant to Rule 7.1 by the International Depositary Authority BCCM™/LMBP identified at the bottom of next page

International Form BCCM™/LMBP/BP/4/99-23

To : Name of the depositor : Janssen Pharmaceutica N.V.

**Address : Turnhoutseweg 30
B-2340 Beerse
Belgium**

I. Identification of the microorganism:

I.1 Identification reference given by the depositor:

VEGF-X CUB PET22b

I.2 Accession number given by the International Depositary Authority:

LMBP 3991

**BELGIAN COORDINATED COLLECTIONS OF MICROORGANISMS - BCCM™
LMBP-COLLECTION**

PCT/US99/30503

Page 1 of Form BCCM™/LMBP/BP/9/99-23 Viability statement

**Budapest Treaty on the International Recognition of the Deposit of Microorganisms for
the Purposes of Patent Procedure**

**Viability statement issued pursuant to Rule 10.2 by the International Depositary
Authority BCCM™/LMBP identified on the following page**

International Form BCCM™/LMBP/BP/9/99-23

To : Party to whom the viability statement is issued:

Name : Dr Filip De Corte

**Address : Janssen Pharmaceutica N.V.
Turnhoutseweg 30
B-2340 Beerse
Belgium**

I. Depositor:

I.1 Name : Janssen Pharmaceutica N.V.

**I.2 Address : Turnhoutseweg 30
B-2340 Beerse
Belgium**

II. Identification of the microorganism:

II.1 Accession number given by the International Depositary Authority:

LMBP 3991

**II.2 Date of the original deposit (or where a new deposit or a transfer has been
made, the most recent relevant date) : December 20, 1999**

III. Viability statement.

The viability of the microorganism identified under II above was tested on

: January 11, 2000

**(Give date. In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent
viability test).**

On that date, the said microorganism was: (mark the applicable box with a cross)

☒ **viable**

☐ **no longer viable**

**BELGIAN COORDINATED COLLECTIONS OF MICROORGANISMS - BCCM™
LMBP-COLLECTION**

Page 2 of Form BCCM™/LMBP/BP/4/99-23 Receipt in the case of an original deposit

II. Scientific description and/or proposed taxonomic designation

The microorganism identified under I above was accompanied by:

(mark with a cross the applicable box(es))

- | | | |
|------------------------------------|---|--|
| - a scientific description | yes <input checked="" type="checkbox"/> | no <input type="checkbox"/> |
| - a proposed taxonomic designation | yes <input type="checkbox"/> | no <input checked="" type="checkbox"/> |

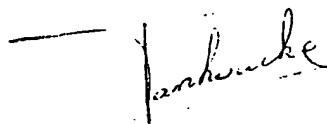
III. Receipt and acceptance

This International Depositary Authority accepts the microorganism identified under I above, which was received by it on (date of original deposit) : December 20, 1999

IV. International Depositary Authority

Belgian Coordinated Collections of Microorganisms (BCCM™)
Laboratorium voor Moleculaire Biologie - Plasmidencollectie (LMBP)
Universiteit Gent
K.L. Ledeganckstraat 35
B-9000 Gent, Belgium

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):



Date : January 12, 2000

Martine Vanhoucke
BCCM/LMBP curator